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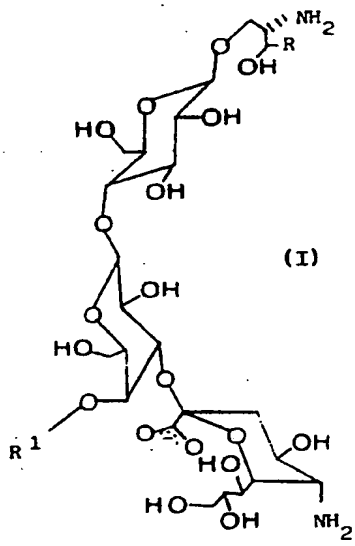

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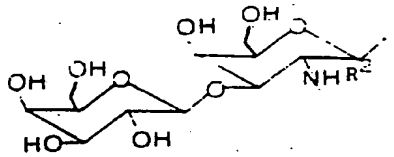

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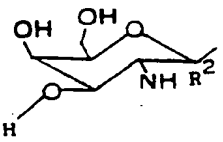

(57) Abstract

The invention relates to N-deacetyl-lysogangliosides and physiologically acceptable salts thereof, especially those of formula (I), wherein R represents $-CH=CH(CH_2)nCH_3$ or $-CH_2CH_2(CH_2)nCH_3$; n is 12 or 14; R^1 represents (II) or hydrogen; and R^2 represents hydrogen or acetyl; for use in therapy. The compounds are useful as inhibitors of phospholipases A_2, and of superoxide production, as antiproliferative and immunosuppressant agents and in the treatment of autoimmune diseases. The invention also relates to the use of N-deacetyl-lysogangliosides for the manufacture of medicaments for the treatment of the aforementioned conditions.

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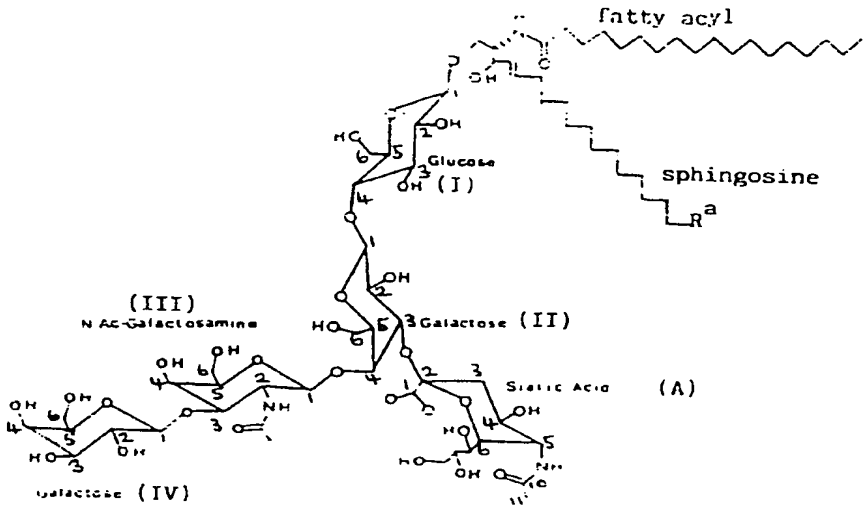
Ganglioside derivatives and their use as medicaments

The present invention relates to ganglioside derivatives, their use as therapeutic agents, methods for the preparation of said derivatives, and pharmaceutical compounds containing them. In particular this invention relates to N-deacylated derivatives of monosialo-gangliosides useful as inhibitors of phospholipases A_2, and of superoxide production, as antiproliferative and immunosuppressant agents and in the treatment of autoimmune diseases.

The gangliosides are a class of naturally occurring glycosphingolipids comprising an oligosaccharide moiety to which may be attached one or more sialic acid groups, and a ceramide portion which contains a sphingosine or sphinganine chain and an acyl moiety derived from a fatty acid. Gangliosides have been found in the brain, spleen, liver, kidneys and blood of mammals and also in chicken eggs (see Ledeen, J. Supramolecular Structure, 8:1-17 (1978) Cell Surface Carbohydrates and Biological Recognition 437-453 for a general review of the gangliosides).

According to the nomenclature proposed by Svennerholm (J. Neurochem., 10, 613, 1963) the various gangliosides are designated by the letter G followed by one of four letters, M, D, T or Q depending on whether the ganglioside is a mono-, di-, tri or tetra-sialo-ganglioside. Thus for example the ganglioside GM_1 is a mono-sialoganglioside with the following structure:



wherein R^a is $-CH_3$ or $-C_3H_7$. GM_1 isolated from natural sources is generally a mixture of molecules wherein R^a is $-CH_3$ and those wherein R^a is $-C_3H_7$ that is having a carbon chain length of 18 or 20 C-atoms. It will be appreciated that the molecule may be in the erythro or threo configuration with respect to the double bond in the sphingosine chain, but the naturally occurring form is the erythro configuration. Naturally occurring GM_1 also contains a small proportion of molecules having a fully saturated sphinganine chain in place of the sphingosine moiety. In addition there is some variation in the length of the fatty acyl chain, with that derived from stearic acid (C_{18}) being the major component; other fatty acyl components include those derived from myristic (C_{14}) arachidic (C_{20}) and lignoceric (C_{24}) acids. (Sonnino et al., Journal of Lipid Research, Vol 26, 1985 p248; and Gazzotti et al., Journal of Neuroscience Research 12:179-192, 1984).

GM_2 has a similar structure but lacks the galactose moiety (IV) of the above structure and GM_3 lacks both the galactose and N-acetyl-galactosamine (III) moieties. The numbering of the hexopyranoside moieties (I) to (IV) and the designation of the sialic acid group (A) are the designations proposed by Sillerud et al., Biochemistry 1982, 21, 1260-1271, and Koerner et al., Biochemistry 1983, 22, 2676-2687 and will be followed hereinafter.

A closely related class of sphingolipids is the lysosphingolipids, also known as lysogangliosides, which lack the fatty acyl moiety of the corresponding ganglioside. It has been reported that lysogangliosides are potent inhibitors of protein kinase C activity and phorbol-diester binding, suggesting they have a role in neurological disorders (Hannun and Bell, Science Vol 235 p670-674). Neuenhofer et al, Biochemistry, (1985) 24 p525-532, describes the synthesis of various lysogangliosides by deacylation of gangliosides GM_2, GM_1 and GD_{1a}, to remove both the fatty acyl moiety and the acetyl group present on the sialic acid residue, followed by reacylation of the sialic acid. The paper also indicates that traces of the completely deacylated GM_1 and GD_{1a} were revealed by FAB mass analysis. No biological activity is ascribed to these compounds.

Sonnino et al. (Journal of Lipid Research, Vol 26, 1985 p248) describe the preparation of various derivatives of GM_1 in which the naturally occurring fatty acyl moiety is replaced with a different fatty acyl moiety. The synthesis of these compounds also proceeds via the lysoganglioside derivative of GM_1 in which the sialic acid moiety is deacetylated. No biological activity is ascribed to these compounds.

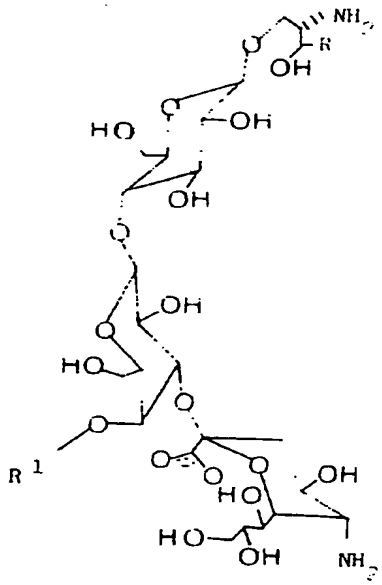
In this specification lysoganglioside derivatives in which the sialic acid residue only is deacetylated will be referred to as mono-N-deacetyl-lysogangliosides e.g mono-N-deacetyl-lyso-GM_1 and lysogangliosides in which both the sialic acid and N-galactosamine residues are deacetylated will be referred to as di-N-deacetyl-lyso-gangliosides. The expression 'N-deacetyl lysoganglioside' will be

used in this specification to mean a mono- or di-N-deacetyl lysoganglioside as well as mixtures thereof. It will be understood that the foregoing terms are not intended to imply any particular route of preparation of these compounds, but they embrace the compounds per se, whether or not they have been prepared by direct deacetylation of the corresponding lysoganglioside GM_1.

It has now been found that certain N-deacetyl-lysogangliosides exhibit therapeutically useful biological activity in a variety of test systems as will be described in more detail hereinafter.

In a first aspect therefore the present invention provides an N-deacetyl lysoganglioside or physiologically acceptable salt thereof for use as a medicament, that is, for use in therapy. Said N-deacetyl-lysoganglioside preferably corresponds to a ganglioside GM_1, GM_2, GM_3 or GD_{1a}.

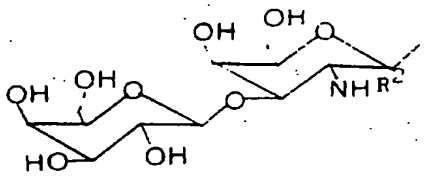
Preferred derivatives for use according to the invention are those of formula (I):

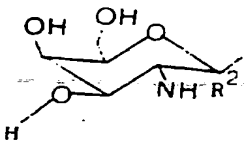


(I)

wherein R represents $-CH=CH(CH_2)nCH_3$ or $-CH_2CH_2(CH_2)nCH_3$;
n is 12 or 14;

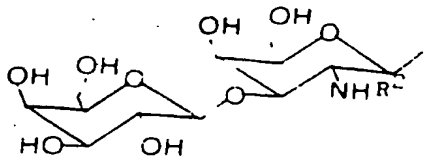
R^1 represents





or hydrogen;
and R^2 represents hydrogen or acetyl;
and physiologically acceptable salts thereof.

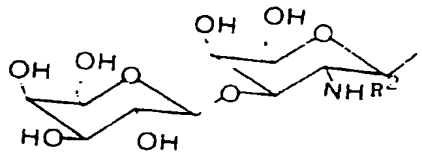
Preferred compounds for use according to the present invention include mono-N-deacetyl-lyso-GM_1 and di-N-deacetyl-lyso GM_1 i.e. the compounds of formula (I) wherein R^1 represents



and R^2 represents respectively acetyl or a hydrogen atom.

Preferably R^2 represents hydrogen.

Further preferred compounds are those of formula (I) wherein n is 14 i.e. those having a C_{20} sphingosine or sphinganine chain. A particularly preferred compound of the present invention is the compound of formula (I) wherein R represents $-CH=CH(CH_2)nCH_3$ or $-CH_2CH_2(CH_2)nCH_3$, n is 14, R^1 is a residue



and R^2 is a hydrogen atom, which compound is herein designated:

di-N-deacetyl-lyso-GM_1-C_{20}.

Preferably R represents $-CH=CH(CH_2)nCH_3$.

Di-N-deacetyl-lyso-GM_1-C_{20} is believed to be a novel compound and as such forms a further aspect of the present invention.

Further novel compounds according to the present invention are the corresponding compound wherein n represents 12, and the corresponding monodeacetyl lysogangliosides, herein respectively designated:

di-N-deacetyl-lyso-GM_1-C_{18};
mono-N-deacetyl-lyso-GM_1-C_{20}; and
mono-N-deacetyl-lyso-GM_1-C_{18}.

Salts of the N-deacetyl lysogangliosides include salts formed with acids and salts formed with bases, and are preferably physiologically acceptable. Suitable acid addition salts include those formed from hydrochloric, hydrobromic, nitric, perchloric, sulphuric, citric, tartaric, phosphoric, lactic, benzoic, glutamic, oxalic, aspartic, pyruvic, acetic, succinic, fumaric, maleic, oxaloacetic, isethionic, stearic, phthalic, methanesulphonic, p-toluene sulphonic, benzenesulphonic, lactobionic and glucuronic acids. Suitable base salts include inorganic base salts such as alkali metal (eg. sodium and potassium) salts and alkaline earth metal (eg. calcium) salts; organic base salts eg. phenylethylbenzylamine, dibenzylethylenediamine, ethanolamine and diethanolamine salts; and amino acid salts eg. lysine and arginine salts.

N-deacetyl-lysogangliosides for use according to the present invention have been found to inhibit phospholipases A_2 (PLA_2), enzymes, both in vitro and in vivo. PLA_2 catalyses the release of arachidonic acid from cell membranes, which acid is then converted in the body to prostaglandins, leukotrienes and thromboxanes. PLA_2 is also involved in generation of platelet activating factor from alkylacetyl phosphatidyl choline. Thus PLA_2 plays a role in a number of conditions including inflammation, thrombotic disorders and allergic disorders. N-deacetyl lysogangliosides have also been found to inhibit superoxide production from human peripheral blood neutrophils in vitro. Superoxide production is believed to be involved in inflammatory processes and reperfusion injury such as occurs in cardiac infarct and kidney ischemia.

N-deacetyl lysogangliosides have alo been found to inhibit the proliferation of leukemia L1210 cells and of Daudi cells in vitro, and to decrease the mortality rate in mice implanted with an experimental Sarcoma 180 tumour. Furthermore it has been found that mice challenged with tumour cells which have been pre-treated in vitro with an N-deacetyl lysoganglioside have an increased survival time as compared with mice challenged with untreated tumour cells, indicating

that N-deacetyl lysogangliosides reduce the oncogenicity of the tumour cells. Mono-N-deacetyl-lyso GM_1 has been demonstrated to have an immunosuppressant effect in an experimental bone marrow transplant in irradiated mice. Without treatment, mice given an allogeneic bone marrow transplant (i.e. bone marrow which is not genetically identical) have a lower survival time (indicating rejection of the bone marrow) than mice given a syngeneic transplant (genetically identical bone marrow), whereas treatment with mono-N-deacetyl-lyso GM_1 has been found to increase the survival time of mice given an allogeneic transplant, in one instance equal to the survival time for a syngeneic transplant. Additionally, it has been found that mono-N-deacetyl-lyso GM_1 exhibits a protective effect on guinea pigs and reduces the mortality rate in the experimental allergic encephalomyelitis test which is a standard animal test model for autoimmune conditions such as multiple sclerosis. (Borel J.F. and Gunn H.C. Annals N.Y. Academy of Science 1986, 475, 307-319).

In a further aspect the present invention provides the use of an N-deacetyl lysoganglioside in the manufacture of a medicament for the treatment of conditions requiring inhibition of phospholipases A_2 or the inhibition of superoxide production, the treatment of a proliferative or autoimmune disease, or suppression of the immune system.

Conditions requiring inhibition of phospholipases A_2 include inflammation for example chronic inflammatory diseases such as arthritis, and inflammatory bowel disease; asthma; and thrombotic disorders for example the prevention of coronary disease or for treatment after an initial heart attack, to prevent subsequent attacks.

Conditions requiring inhibition of superoxide production include reperfusion injury for example in cardiac infarct and kidney ischemia.

Proliferative diseases include various forms of cancer, including leukemias, lymphomas, sarcomas and solid tumours; basal and squamous cell carcinomas of the skin; and psoriasis.

Conditions requiring suppression of the immune system include for example preventing rejection of transplanted organs, including heart, liver, kidneys, bone marrow and endocrine glands. Autoimmune diseases, which are characterised by altered immunological reactivity and manifestations of autoimmunity, include collagen-vascular disorders, such as systemic lupus erythematosus (SLE), necrotising vasculitis, scleroderma, polymyositis, and rheumatoid arthritis; regional enteritis; ulcerative colitis; chronic active hepatitis; glomerulonephritis; Goodpasture's syndrome; autoimmune hemolytic anaemia; idiopathic thrombocytopenic purpura; pemphigus vulgaris; pemphigoid; primary myxoedema; Hashimoto's thyroiditis; thyrotoxicosis; pernicious anaemia; autoimmune atrophic gastritis; Addison's disease; juvenile diabetes; myasthenia gravis; sympathetic ophthalmia; phacogenic uveitis; multiple sclerosis; ideopathic leucopenia; primary biliary cirrhosis; Sjogren's syndrome; dermatomyositis and discoid LE.

The amount of N-deacetyl lysoganglioside required to be effective as a medicament will, of course, vary and is ultimately at the discretion of the medical or veterinary practitioner. The factors to be considered include the condition being treated, the route of administration, and nature of the formulation, the mammal's body weight, age and general condition, and the particular compound to be administered. A suitable therapeutic dose is in the range of about 0.0005 to about 10mg/kg bodyweight, eg 0.01 to about 5mg/kg body weight, preferably in the range of about 0.1 to 2mg/kg. The total daily dose may be given as a single dose, multiple doses, e.g., two to six times per day or by intravenous infusion for selected duration. For example, for a 75 kg mammal, the dose range for would be about 0.5 to 500mg per day eg. 10 to 250mg per day, and a typical dose could be about 100mg per day. If discrete multiple doses are indicated

treatment might typically be 25mg of a compound of formula (I) given up to 4 times per day. A suitable effective dose for anti-tumour treatment is in the range of about 0.1 to about 10mg/kg bodyweight eg 1 to 5mg/kg. For anti-tumour treatment a suitable dose range for a 75kg mammal is 10 to 750mg per day, eg. 50 to 500mg per day.

Whilst it is possible for an N-deacetyl lysoganglioside according to the invention to be administered alone, it is preferable to present said compound in a pharmaceutical formulation. Formulations of the present invention for medical use comprise an N-deacetyl lysoganglioside or a physiologically acceptable salt thereof together with one or more pharmaceutically acceptable carriers and optionally other therapeutic ingredients. The carrier(s) should be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The present invention, therefore, further provides a pharmaceutical formulation comprising an N-deacetyl lysoganglioside or a physiologically acceptable salt thereof together with a pharmaceutically acceptable carrier therefor.

There is also provided a method for the preparation of a pharmaceutical formulation comprising bringing into association an N-deacetyl lysoganglioside or a physiologically acceptable salt thereof, and a pharmaceutically acceptable carrier therefor.

Formulations according to the present invention include those suitable for oral, topical, rectal or parenteral (including subcutaneous, intramuscular and intravenous) administration. Preferred formulations are those suitable for oral or parenteral administration. For treatment of solid tumours an N-deacetyl lysoganglioside may also be formulated for percutaneous administration around the perimeter of the tumour.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the N-deacetyl lysoganglioside into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing said compound into association with a liquid carrier or a finely divided solid carrier or both and then, if necessary, shaping the product into desired formulations.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the N-deacetyl lysoganglioside; as a powder or granules; or a solution or suspension in an aqueous or non-aqueous liquid such as a syrup, an elixir, an emulsion or a draught.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the N-deacetyl lysoganglioside in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound with any suitable carrier.

A syrup may be made by adding the N-deacetyl lysoganglioside to a concentrated, aqueous solution of a sugar, for example sucrose, to which may also be added any accessory ingredients. Such accessory ingredient(s) may include flavourings, an agent to retard crystallization of the sugar or an agent to increase the solubility of any other ingredients, such as a polyhydric alcohol for example glycerol or sorbitol.

Formulations for rectal administration may be presented as a suppository with a conventional carrier such as cocoa butter.

Formulations suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the N-deacetyl lysoganglioside which is preferably isotonic with the blood of the recipient.

Useful formulations also comprise concentrated solutions or solids containing an N-deacetyl lysoganglioside which upon dilution with an appropriate solvent give a solution for parenteral administration as above.

In addition to the aforementioned ingredients, the formulations of this invention may further include one or more accessory ingredient(s) selected from diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like.

The N-deacetyl lysogangliosides for use according to the present invention may be administered in combination with other therapeutic agents, for example in a combined pharmaceutical formulation, or concurrently with other agents as part of the same treatment regimen. Such other therapeutic agents include for example other immunosuppressants such as cyclophosphamide; antitumour agents; corticosteroids; cytokines; non-steroidal anti-inflammatory agents; antihistamine agents; antiserotonin agents and antiplatelet agents.

The N-deacetyl-lysogangliosides for use according to the present invention may be prepared from a sialoganglioside by subjecting said sialoganglioside to alkaline hydrolysis in a suitable solvent, for example an alcohol such as a C_{1-4} alkanol, and at an elevated temperature, conveniently in the range 100-130°C, or by using enzymatic hydrolysis. The alkaline hydrolysis may be effected by methods well known in the art, conveniently using aqueous sodium or potassium hydroxide or tetramethylammonium hydroxide in admixture with a C_{1-4} alkanol, eg. methanol or n-butanol. The hydrolysis may be conveniently carried out at reflux temperature. The duration of

hydrolysis will depend on the other conditions used, but in general will be at least 3 hours and may continue for up to 30 hours. As will be readily apparent to those skilled in the art, the level of deacylation achieved will depend upon the precise combination of reaction conditions used and if a complete deacylation is required, to produce a di-N-deacetyl-lysoganglioside, more stringent conditions should be chosen than are used to effect a partial de-acylation of the starting material. It will also be appreciated that the reaction can be monitored for example by thin layer chromatography or by spectroscopic analysis of the reaction mixture, and the end point thus determined. The N-deacetyl lysoganglioside may subsequently be obtained from the reaction mixture by methods well-known in the art e.g by removing the organic solvent and dialysis against water, conveniently for a period between 24 and 72 hours, preferably 48 hours. Either distilled or natural water may be used in this step. The resulting solution may be freeze-dried or subjected to further purification, eg. by column chromatography. If the product contains a mixture of mono-and di-N-deacetyl lysogangliosides these may be separated by column chromatography.

Sialogangliosides which may be employed as starting materials in the above process are preferably monosialogangliosides and include GM_1, GM_2, GM_3 and GD_{1a}. It will be appreciated that the nature of the fatty acyl moiety in the starting material is not of importance, as this moiety is not present in the final product. Preferably the ganglioside starting material is in the naturally-occuring <u>erythro</u> form.

The N-deacetyl lysoganglioside derivatives obtainable according to the above process will usually contain less than 50%, preferably less than 30% and most preferably less than 10% of impurities, such as the ganglioside starting material and other ganglioside derivatives. Purification of the N-deacetyl lysoganglioside initially obtained may be effected by methods well known in the art, such as column chromatography.

In a further aspect of the invention there is provided a mono-or di-N-deacetyl lysoganglioside GM_1 characterised in that it is at least 50%, preferably at least 70% e.g. at least 90% pure.

It will be appreciated that where the starting material comprises a mixture of homologous compounds, such as GM_1 or GD_{1a} which each exists as a mixture of the compound wherein n is 12 and wherein n is 14, the corresponding N-deacetyl lysoganglioside may also be a mixture of homologues. The purity of the final compound is calculated with reference to such mixtures, and not to any one homologue. The ratio of homologues obtained in the preparation of the N-deacetyl lysogangliosides may vary within wide limits. In practice however it is found to be close to a 1:1 mixture. Similarly, where a proportion of the starting ganglioside is in the sphinganine form the final compound will also contain a proportion of sphinganine moieties.

An N-deacetyl lysoganglioside containing both C_{18} and C_{20} components, (i.e. a mixture of compounds wherein n is 12 and 14) can be separated by chromatographic methods to give the individual C_{18} and C_{20} components. Alternatively, the ganglioside starting material, such as GM_1 may first be separated for example by chromatographic methods, into its C_{18} and C_{20} components, which can then be used to prepare the corresponding C_{18} and C_{20} N-deacetyl lysogangliosides according to the general method described hereinabove. The C_{18} component of the starting ganglioside is preferably eluted using an aqueous organic solvent and the C_{20} component is preferably eluted with a non-aqueous organic solvent.

The C_{18} and C_{20} N-deacetyl lysoganglioside derivatives obtainable according to the above processes will usually contain less than 50%, preferably less than 30% and most preferably less than 10% of impurities such as ganglioside starting material or other ganglioside derivatives. In a further aspect of the invention there is provided a C_{18} or C_{20} mono- or di- N-deacetyl lysoganglioside characterised in that it is in substantially pure form, for example at least 50%,

preferably at least 70% eg. at least 90% pure. Preferably the said C_{18} or C_{20} N-deacetyl lysoganglioside is selected from

mono-N-deacetyl-lyso GM_1-C_{20}
mono-N-deacetyl-lyso GM_1-C_{18}
di-N-deacetyl-lyso GM_1-C_{20}
di-N-deacetyl-lyso GM_1-C_{18}

in substantially pure form.

The invention will now be illustrated by the following non-limiting examples:

Example 1

Preparation of Mono-N-deacetyl-lyso GM_1 and di-N-deacetyl-lyso GM_1

150mg of GM_1 (Opocrin, purity 98% by HPLC and t.l.c.) were hydrolyzed by refluxing at 115-117°C for 3.5 hours with 10ml of a mixture of 10 M KOH and butan-1-ol(1/9 v/v). After cooling at room temperature overnight, 10ml of water were added to the hydrolysis solution and the carefully mixed solution was placed in a separation funnel overnight. The lower water phase which contained lysoganglioside was isolated, concentrated under vacuum to eliminate butanol traces, then adjusted to pH9 with HCl(12N) and clarified by centrifugation (3000 rpm) for 15 minutes. The clear surnatant was dialysed in a Visking tube against 10 l of distilled water (changed 3 times per day) for two days then freeze-dried, to give the hydrolysis product. This was purified by column chromatography on silica gel Si60 (Merck art.9385), using the following mobile phase scheme:

Eluant A: Butan -1-ol/methanol/water (2:2:0.75 v/v/v) 1200 ml
Eluant B: Butan -1-ol/methanol/water (2:2:1 v/v/v) 500 ml

1g of the hydrolysis product was dissolved in 60ml of eluant A and sonicated at 45^{0}C for 60 minutes, followed by filtration through glass wool to eliminate residual insoluble matter. The solution was then chromatographed at a flow rate of 20ml/min and at 45^{0}C (water jacket). 50ml fractions of each eluant were collected and concentrated under vacuum at 40^{0}C.

Fractions from eluant A gave mono-N-deacetyl-lyso GM_1 as a white fluffy power. Fractions from eluant B gave di-N-deacetyl-lyso GM_1 as a white fluffy powder.

Physical Characteristics

mono-N-deacetyl-lyso GM_1

Rf = 0.13 (Plates: Kieselgel 60 TLC F254 (Merck art. 5715)20x20 cm - 0.25 mm, activated by heating at 100^{0}C for 30 minutes and cooled to room temperature just before use. After 1 hour of saturation the plates were developed with chloroform:methanol:2.5M ammonia (50:40:10), as eluant. Detection: 1) Resorcinol, 2) Orcinol).

Preparation of Orcinol Spray:

Solution A: dissolve 1g iron chloride in 100ml 10% sulphuric acid.
Solution B: 6% ethanolic orcinol solution.
Mix 10ml A and 1ml B immediately before use. After spraying, the plates are heated for 20-25 minutes at 100^{0}C.
Loading: 2011.
Run length: 12.5cm.

Preparation of Resorcinol Spray:

2g of resorcinol was dissolved in 100ml of distilled water. 10ml of this solution was added to 80ml of concentrated hydrochloric acid

containing 0.25ml of 0.1M copper sulphate. The volume of the reagent was made up to 100ml of distilled water. The reagent was prepared at least 4 hours before use.

The ^{1}H nmr spectrum obtained on the product was substantially identical to that shown in Figure 1.

Di-N-deacetyl-lyso-GM_1

RF=0.05 (Plates and Detection as above) ^{1}H nmr and ^{13}C nmr-the spectra obtained were substantially identical to those shown in Figures 2(a) and 2(b).

Example 2

Preparation of gangliosides GM_1-C_{18} and GM_1-C_{20}

Eluents

Eluent A :
water:tetrahydrofuran:methanol;16:1:83v/v/v
Eluent B :
water:tetrahydrofuran:methanol;8:8:84v/v/v
Eluent C :
tetrahydrofuran:methanol;10:90v/v

2.5g. of GM_1 (Opocrin) were dissolved in 200ml of eluent A. The solution was filtered through glass wool to eliminate insoluble material and loaded onto a column (Jobin Yon, diameter 4cm, height 50cm) packed with silica (200g; RP18 25-40lm, art 9303 Merck). The column was eluted successively with Eluent A (3000ml), Eluent B (800ml) and Eluent C (800ml), at a flow rate of 25-30ml/min and at room temperature. 200ml fractions of each eluent were collected and

concentrated under vacuum at 40°C. The purity of each fraction was checked by HPLC. Fractions 2-5 gave ganglioside GM_1-C_{18} as a powder (50-120mg/fraction); fractions 6-10 gave a mixture of GM_1-C_{18} and GM_1-C_{20} as a powder (50-120mg/fraction); fractions 11-18 gave GM_1-C_{20}. Values from the ^1H nmr spectra obtained on GM_1-C_{20} are given in Table 1 below. The 13C nmr spectrum obtained on GM_1-C_{20} was substantially identical to that shown in Figure 3. The 13C spectrum obtained on GM_1-C_{18} was substantially identical to that shown in Figure 4.

Example 3

a) Preparation of di-N-deacetyl-lyso-GM_1-C_{20}

3 g. of GM_1 C_{20} were hydrolysed by reflux boiling (temperature 118-121°C) for 12 hours with 1 L of a mixture of 10M KOH and butan-1-ol [1/9 v/v]. After cooling at room temperature, the reaction mixture was slowly neutralized by several mls of HCl 37% until a white precipitate formed, which was allowed to settle for ca. 1 hour. After removal of the upper phase, the precipitate was suspended in 300mL of distilled water, and reneutralized. Traces of organic solvent were removed under vacuum and the cloudy suspension was dialyzed in a visking tube against 12 L of distilled water (two changes per day for 2 days) and freeze-dried. The freeze dried powder was suspended with 250 mL of distilled water, filtered through a filter paper and washed twice with 100 mL of H_2O, to remove any water-insoluble material.

The combined filtrate and washings were filtered through a 0.2l membrane filter and freeze-dried, to give the title compound.

Values from the ^1H nmr spectrum obtained on di-N-deacetyl-lyso-GM_1-C_{20} are given in Table 1 below. The ^1H nmr spectrum is shown in Figure 5.

b) Preparation of mono-N-deacetyl-lyso-GM_1-C_{20}

GM_1-C_{20} was hydrolysed according to the method of Example 3(a), but the reaction was terminated after 3.50 hours. At this atage the product was found to contain a mixture of mono- and-di-N-deacetyl lyso GM_1-C_{20}, the major component being the monodeacetyl compound. The mixture was separated by column chromatography to give the title compound.

The ^1H nmr spectrum is reproduced as Figure 6.

TABLE 1

	Product of Example 2	Product of Example 3a
R4	5.34dd;J4,5=15.1 J3,4= 6.6	5.43dd;J4,5=15.1 J3,4= 6.8
R5	5.53dt;J5,6=7.1	5.59dt;J5,6=6.9
III-1	4.82d;J1,2=8.5	4.87d;J1,2=8.4
II-1	4.28d;J1,2=7.7	4.38d;J1,2=7.7
IV-1	4.23d;J1,2=7.0	4.29d;J1,2=7.3
I-1	4.15d;J1,2=7.8	4.18d;J1,2=7.8
A6	3.11d;J=9.6	3.12d;J=10.3
A3a	1.63t;J3e,3a=12.4	1.54t;J=12.1
R6	1.93bs	1.98q;J=6.9
R8	2.02t;J=7.3	-
A11	1.88s	-
III-8	1.74s	-
R10	1.23bs	1.23bs
R14	0.85;J=6.9	0.85t;J=6.9

The numbering in Table 1 follows that proposed by Sillerud et al., Biochemistry 1982, 21, 1260-1271 and Koerner et al., Biochemistry 1983, 22, 2676-2687 as shown hereinbefore.

Example 4

a) Preparation of di-N-deacetyl-lyso-GM_1-C_{18}

The title compound was prepared in a similar manner to Example 3(a).

b) mono-N-deacetyl-lyso-GM_1-C_{18}

The title compound was prepared using a similar procedure to Example 3(b). The ^1H nmr spectrum of the product is reproduced as Figure 7.

Biological test results

Example 5

Effect of mono-N-deacetyl-lyso-GM_1 on Proliferation of L1210 and Daudi cells in vitro

Method

Proliferation assay: The cell lines were maintained in RPMI 1640 medium with 25mM HEPES buffer and L-Glutamine (GIBCO 041-02400M) supplemented with antibiotics and 10% Foetal Calf Serum Inactivated GIBCO 013-06290H). Tumor cells were washed and diluted with complete medium at the appropriate dilutions and added (2ml) to wells of FALCON 3047 plate. All drugs in various concentrations, diluted in complete

medium, were added to triplicate wells in a volume of 0.2ml. The cells were incubated at 37°C in 5% CO_2 atmosphere for 24,48 and 72 hours depending on the particular experiment. After incubation, cells were counted using a cellular counter (COULTER COUNTER ZM).

Test Compound

Interferon	
gamma	1000 U/ml
beta	1000 U/ml
alpha	1000 U/ml
mono-N-deacetyl-lyso-GM_1	100mcg/ml

Results

The results which are presented graphically in Figures 8 and 9 and numerically in Table 2 demonstrate that mono-N-deacetyl-lyso-GM_1 significantly reduced the proliferation of L1210 and Daudi cells in vitro, both as compared with untreated controls and with various interferons.

TABLE 2

L1210 ($X10^3$)	48 hours mean (S.E)	%	72 hours mean (S.E)	%
Controls	346 (37)		1702 (41.76)	-
gamma interferon 1000U/ml	336 (61.6)	-2.89	1102 (32.4)	-35
beta interferon 1000U/ml	310 (12.2)	-10.4	1059 (24)	-38
alpha interferon 1000U/ml	294 (7.86)	-15.02	1114 (38)	-34
mono-N-deacetyl-lyso-GM_1	177 (4)	-48.84	527 71.5	-71.5

TABLE 2 (CONTINUED)

DAUDI (X103)	48 hours mean (S.E)	%	72 hours mean (S.E)	%
Controls	1250 (39.5)	-	1984 (76.9)	-
gamma interferon 1000U/ml	1212 (69.2)	-3.04	2209 (44.7)	11.3
beta interferon 1000U/ml	1151 (42.2)	-7.92	2179 (34.8)	9.8
alpha interferon 1000U/ml	1121 (30.9)	-10.3	2157 (147)	8.7
mono-N-deacetyl-lyso-GM_1 100mcg/ml	662 (55.2)	-47	918 (48)	-53

Example 6

Effect of mono-N-deacetyl-lyso-GM_1 on Sarcoma 180 *in vivo*

Method

Groups of 10 mice (male, CD1, Carles River) were implanted subcutaneously in the right flank on day 0 with Sarcoma 180 (NCI G01143), 1.6x 10^5 cells/mouse suspended in PBS Dulbecco's (GIBCO 041-04190H). Drugs were adminsitered by perilesional injection on

days 0, 2, 4, 7, 9 and 11. Two groups of mice received mono-N-deacetyl-lyso-GM_1 at 100 and 10mg/mouse and two groups received Glucan (Sigma G-5011) solubilised in FBS, also at 100 and 10 mcg/mouse.

Results

The results presented in Table 3 demonstrate that mice treated with mono-N-deacetyl-lyso-GM_1 were significantly protected as indicated by the fact that no deaths occurred up to 30 days from implantation of the tumour cells.

TABLE 3

Groups	Mice	15th day % of Mortality	30th day % of Mortality
Controls	10	30	60
mono-N-deacetyl-lyso-GM_1 100mcg/mouse	10	0	0
mono-N-deacetyl-lyso-GM_1 10mcg/mouse	10	0	0
Glucan 100mcg/mouse	10	10	30
Glucan 10mcg/mouse	10	10	10

Example 7

a) Effect of pre-incubation of Lewis Lung Carcinoma Cells with mono-N-deacetyl-lyso-GM_1

Method

Lewis Lung Carcinoma Cells ($1x10^6$ cells/ml) were incubated for 2 hours at 37°C and 5% CO_2, with mono-N-deacetyl-lyso-GM_1 at concentrations of 200mcg/ml, 100mcg/ml and 50mcg/ml. Groups of mice (C57BL6, male, Charles River) were challenged with the treated cells (0.1ml/mouse i.m.). A control group received a challenge of untreated cells. The mice were observed and the time to death recorded.

Results

The time to death increased with the amount of mono-N-deacetyl-lyso-GM_1 used to pre-treat the cells, indicating that mono-N-deacetyl-lyso-GM_1 directly reduces the oncogenicity of the cells. The results are presented graphically in Figure 10.

b) Effect of preincubation of Lymphoma L5178Y Cells with mono-N-deacetyl-lyso-GM_1

Method

Lymphoma L5178Y cells ($6x10^5$ cells/ml) were incubated for 90 minutes at 37°C and 5% CO_2, with mono-N-deacetyl-lyso-GM_1 at concentrations of 100 and 50mcg/ml. Groups of mice (CDF1, male, Charles River) were challenged with the treated cells

(0.1ml/mouse i.p.). A control group received a challenge of untreated cells. The time to death was recorded.

Results

The time to death increased with the amount of mono-N-deacetyl-lyso-GM_1 used to pre-treat the cells, indicating that mono-N-deacetyl-lyso-GM_1 directly reduces the oncognecity of these cells. The results are presented graphically in Figure 11.

Example 8

Effect of mono-N-deacetyl-lyso GM_1 on Bone Marrow Graft Rejection in Irradicated Mice

Method

Three groups of mice (C57BL/6, male, 18-20g) were given gamma irradiation, 10Gy, at day-1. One group was given a syngeneic bone marrow graft from non-irradiated C57 BL/6 mice ($1x10^6$, i.v.) at day 0. Two groups were given an allogeneic bone marrow graft from C3H male mice, ($1x10^6$, i.v.) at day 0. One of these "allogeneic groups" was treated with mono-N-deacetyl-lyso-GM_1 1mg/kg i.e. at days 0, 1, 2, 3 and 4 after transplantation. A control group was irradiated but given no bone marrow graft. The mice were observed until the 35th day after transplantation and the mean survival time (MST) calculated.

Results

The results of two experiments which are presented in Tables 4 and 5 and graphically in Figures 12 and 13 demonstrate that mono-N-deacetyl-lyso-GM_1 increases the survival time of mice given an allogeneic bone marrow graft, indicating that mono-N-deacetyl-lyso-GM_1 prevents rejection of the graft.

TABLE 4

Groups	MST	% of mortality
Controls	14.7	85.7
Syngeneic	29.1	7.1
Allogeneic	23.0	57.1
Allogeneic + mono-N-deacetyl-lyso-GM_1	30.7	7.1

No of animals: 14 per group

TABLE 5

Groups	No of animals	MST
Controls	12	11.0
Syngeneic	12	25.5
Allogeneic	14	5.0
Allogeneic + mono-N-deacetyl-lyso-GM_1	15	13.4

Example 9

Effect of mono-N-deacetyl-lyso-GM_1 in Experimental Allergic Encephalomyelitis

Method

Antigen solution was prepared as follows:-

Myelin basic protein from bovine brain M 1891 SIGMA St. Louis, USA, was dissolved in saline at a concentration of 5mg/ml and mixed 1:1 with Freund's complete adjuvant (FCA - Difco cod 0638-60-7, Detroit USA), and 8mg/ml of M tuberculosis H37 Ra - Difco cod 3114-33-8, Detroit USA).

Two groups of female outbred Hartley guinea pigs, eight per group, were injected with 0.1ml of antigen solution into each hind footpad on day 0, to induce experimental allergic encephalomyelitis (EAE). One of these groups was treated daily for 5 days before challenge and for 3 days after with mono-N-deacetyl-lyso-GM_1, 5mg/kg per day, i.p. A control group received FCA only.

Clinical assessment of EAE - The animals were weighed and observed daily from the first injection. The severity of EAE was graded 1 to 5 according to the criteria described by Keith & Mc Dermott, J. Jeurol Sci 1980, 46:353-364.

Clinical grading and criteria for relapse -
0 - No clinical signs; 1 - Weight loss; 2 - Mild paresis; 3 - Moderate paresis; 4 - Severe paresis and faecal impaction; 5 - Death.

Surviving animals were euthanised by gassing, brains were fixed in 4% paraformaldehyde for histological scores, and spleens were weighed and compared to controls.

Results

The results, which are shown in Tables 6 and 7 and Figures 14 and 15 demonstrate that mono-N-deacetyl-lyso-GM_1 reduces the clinical symptoms of EAE and increases the mortality rate.

TABLE 6

	Mortalities (dead / total)								
	Days								
Groups	10	11	12	13	14	15	16	17	18
Controls	0/8	0/8	1/8	4/8	4/8	4/8	4/8	4/8	4/8
mono-N-deacetyl-lyso-GM_1	0/8	0/8	0/8	1/8	1/8	1/8	2/8	2/8	2/8

TABLE 7

Results of effect of mono-N-deacetyl-lyso-GM_1 treatment on clinical assessment of EAE.

Days	0	7	14	21	28
Controls	0	3	21	29	35
mono-N-deacetyl-lyso-GM_1	0	0	9	19	20

Example 10

Effect of di-N-deacetyl-lyso-GM_1-C_{20} on phospholipases A_2 (PLA_2) in vitro

Di-N-deacetyl-lyso-GM_1-C_{20} was tested by the method of Kremer et al, Biochemistry, 16, 3932, 1977 and found to exhibit an inhibitory effect on hog pancreas PLA_2 on liposomes. Results are presented in Figure 16 below.

Example 11

Effect of di-N-deacetyl-lyso-GM_1-C_{20} on rabbit platelet aggregation in vivo

Method

Preparation of Platelet suspension (Platelet Rich Plasma)

Blood samples were collected from fasted New Zealand rabbits by cardiac puncture in plastic tubes containing the anticoagulant sodium citrate (final concentration 0.38%). Platelet Rich Plasma (PRP) was prepared at room temperature by centrifugation at 150g for 20 min. and was then stored at room temperature. Platelet concentration was adjusted to 2.5 to 3 x 10^8/ml.

Aggregometric Studies

Platelet aggregation was studied by the turbidimetric method of Born (Nature 194:927,1962) at a constant temperature of 37#C. Platelet suspension sample (1ml) were stirred in the Born aggregometer and the light transmission was monitored by continuous recording (Servogor 2s,

Goers). The aggregating agent used was : Sodium arachidonate (Fluka) at the final concentrations of 20-80 mM.

Results

The results, which are presented graphically in Figure 17 indicate that the compound inhibits platelet aggregation in rabbits, using arachidonic acid as agonist at doses of 0.2 and 0.13mg/kg p.o.

Example 12

Effect of Di-N-deacetyl-lyso-GM_1-C_{20} on bleeding time in mice

Method

Di-N-deacetyl-lyso-GM_1-C_{20} was administered at a dose of 0.1mg/kg, orally to male, CD1 mice (C. River, Como, Italy) weighing 22-24g at 96, 72, 48 and 24 hours prior to testing. The mice were anaesthetised and placed in a plastic mice restrainer (Harvard 52-0882) with several openings from one of which the animal's tail emerged. Bleeding was caused by transection of the tail, 5-6mm from the tip using a disposable surgical blade (N#18-Martin). The tails were kept vertically and placed in isotonic solution (saline) at 37#C immediately after the cut. Time in seconds was measured from the moment of the tail cut until bleeding stopped completely (no rebleeding for at least 30 sec.). Student's t-test was used for statistical analysis.

Reference : G.B.Gervasi, C.Bartoli, G.Carpita and M.Baldacci Arzneim Forsch./Drug Res.(1988) 38(II) N#9,1268-1270.

	Animals	mean(sec.)	% S.E.	% Incr.	P<
Control	19	77.16	15.3	-	-
Di-N-deacetyl-lyso-GM_1-C_{20}	19	180.5	42	134	0.029

Example 13

Effect of Di-N-deacetyl-lyso-GM_1-C_{20} on thrombin-induced thromboembolism in mice

Method

Di-N-deacetyl-lyso-GM_1-C_{20} was tested according to the method of Gomi et al. (Blood Vol.75 No.7 1990 pp1396-1399).

The results, which are presented in figure 18 show that the test compound significantly reduces the mortality of the mice.

Example 14

The following represent examples of formulations which may be prepared according to the present invention.

A. TABLET

Compound of Formula I	100.0 mg
Pregelatinised Corn Starch	60.0 mg
Sodium Starch Glycollate	20.0 mg
Magnesium Stearate	4.0 mg

The Compound of formula (I) is finely ground and intimately mixed with the powdered excipients, pregelatinised corn starch and sodium starch glycollate. The powders are wetted with purified water to form granules. The granules are dried and mixed with the magnesium stearate. The formulation is then compressed into tablets weighing approximately 184 mg each.

B. TABLET

Compound of formula (I)	100.0 mg
Sodium Starch Glycollate	20.0 mg
Lactose	83.8 mg
Magnesium Stearate	4.2 mg
Polyvinylpyrrolidone	14.0 mg

The Compound of formula (I) is finely ground and intimately mixed with the powdered excipients, sodium starch glycollate and lactose. The powders are wetted with a solution of polyvinylpyrrolidone dissolved in purified water and denatured alcohol to form granules. The granules are dried and mixed with the magnesium stearate. The formulation is then compressed into tablets weighing approximately 222 mg each.

C. CAPSULES

Compound of formula (I)		100.0 mg
Corn Starch		50.0 mg
Magnesium Stearate		3.0 mg

The finely divided compound of formula (I) is mixed with powdered corn starch. The dried powder is mixed with magnesium stearate and filled into hard-shell gelatin capsules.

D. SUSPENSION

Compound of formula (I)		100.0 mg
Dispersible Cellulose		100.0 mg
Glycerin		500.0 mg
Sucrose		3,500.0 mg
Flavouring Agent		q.s.
Colouring Agent		q.s.
Preserving Agent		0.1%
Purified Water	q.s. to	5.0 ml

The compound of formula (I) is suspended in the glycerin and a portion of the purified water. The sucrose and preserving agent are dissolved in another portion of hot purified water, and then the colouring agent is added and dissolved, followed by the dispersible cellulose. The two preparations are mixed and cooled before the flavouring agent is added. Purified water is added to final volume. The resulting suspension is throughly mixed.

E. IV INJECTION

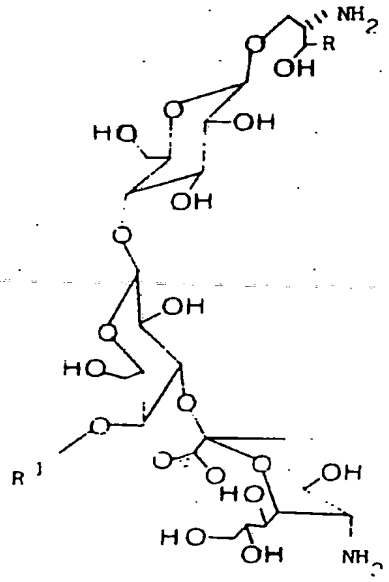
Compound of formula (I)	5.0 mg
Hydrochloric Acid	as needed for pH adjustment
Water for Injections	q.s. to 10 ml

The compound of formula (I) is added to a portion of the Water for Injections. The pH is adjusted with hydrochloric acid to dissolve the compound. Water for Injections is added to final volume and solution is complete after thorough mixing. The solution is sterilised by filtration through a 0.22 micrometer membrane filter and aseptically filled into sterile 10 ml ampoules or vials.

CLAIMS

1. An N-deacetyl-lysoganglioside or a physiologically acceptable salt thereof for use in therapy.

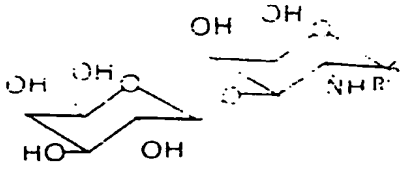
2. An N-deacetyl-lysoganglioside for use according to claim 1, wherein said N-deacetyl-lysoganglioside has the formula (I):

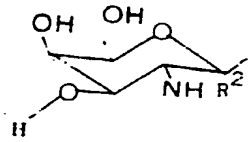


(I)

wherein R represents $-CH=CH(CH_2)nCH_3$ or $-CH_2CH_2(CH_2)nCH_3$;
n is 12 or 14;

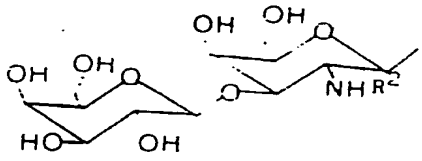
R^1 represents





or hydrogen;
and R^2 represents hydrogen or acetyl;
or a physiologically acceptable salt thereof.

3. An N-deacetyl-lysoganglioside or a physiologically acceptable salt thereof according to claim 2 wherein R^1 represents



and R^2 represents respectively acetyl or a hydrogen atom.

4. N-deacetyl-lysoganglioside or a physiologically acceptable salt thereof according to claim 2 or claim 3 wherein R^2 represents hydrogen.

5. An N-deacetyl-lysoganglioside or a physiologically acceptable salt thereof according to any of claims 2 to 4 wherein R represents $-CH=CH(CH_2)nCH_3$ or $-CH_2CH_2(CH_2)nCH_3$ and n is 14.

6. An N-deacetyl-lysoganglioside or a physiologically acceptable salt thereof according to any of claims 1 to 5 wherein R is $-CH=CH(CH_2)_{14}CH_3$.

7. A mono- or di- N-deacetyl-lysoganglioside GM_1 or a physiologically acceptable salt thereof in substantially pure form.

8. di-N-deacetyl-lyso-GM_1C_{20};
di-N-deacetyl-lyso-GM_1-C_{18};
mono-N-deacetyl-lyso-GM_1-C_{20};
mono-N-deacetyl-lyso-GM_1-C_{18}; or a physiologically acceptable salt thereof.

9. A pharmaceutical composition comprising a compound as defined in any of claims 1 to 8 or a physiologically acceptable salt thereof together with a pharmaceutically acceptable carrier therefor.

10. A pharmaceutical composition according to claim 9, which includes a further therapeutic agent.

11. Use of a compound as defined in any of claims 1 to 8 or a physiologically acceptable salt thereof for the manufacture of a medicament for the treatment of conditions requiring inhibition of phospholipases A_2.

12. Use of a compound as defined in any of claims 1 to 8 or a physiologically acceptable salt thereof for the manufacture of a medicament for the treatment of conditions requiring inhibition of superoxide production.

13. Use of a compound as defined in any of claims 1 to 8 or a physiologically acceptable salt thereof for the manufacture of a medicament for the treatment of proliferative diseases.

14. Use of a compound as defined in any of claims 1 to 8 or a physiologically acceptable salt thereof for the manufacture of a medicament for the treatment of autoimmune diseases.

15. Use of a compound as defined in any of claims 1 to 8 or a physiologically acceptable salt thereof for the manufacture of a medicament for suppression of the immune system.

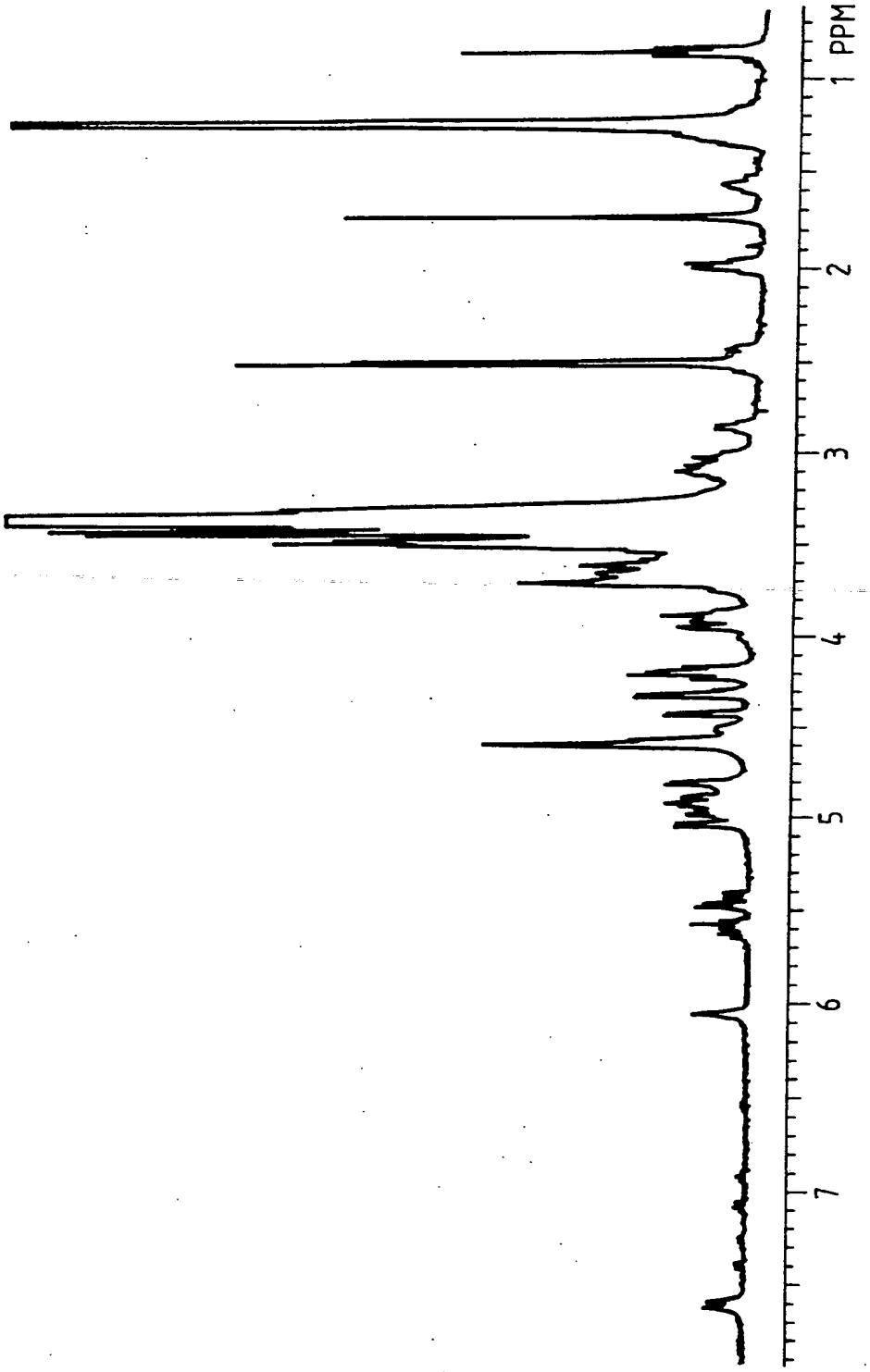


Fig. 1.

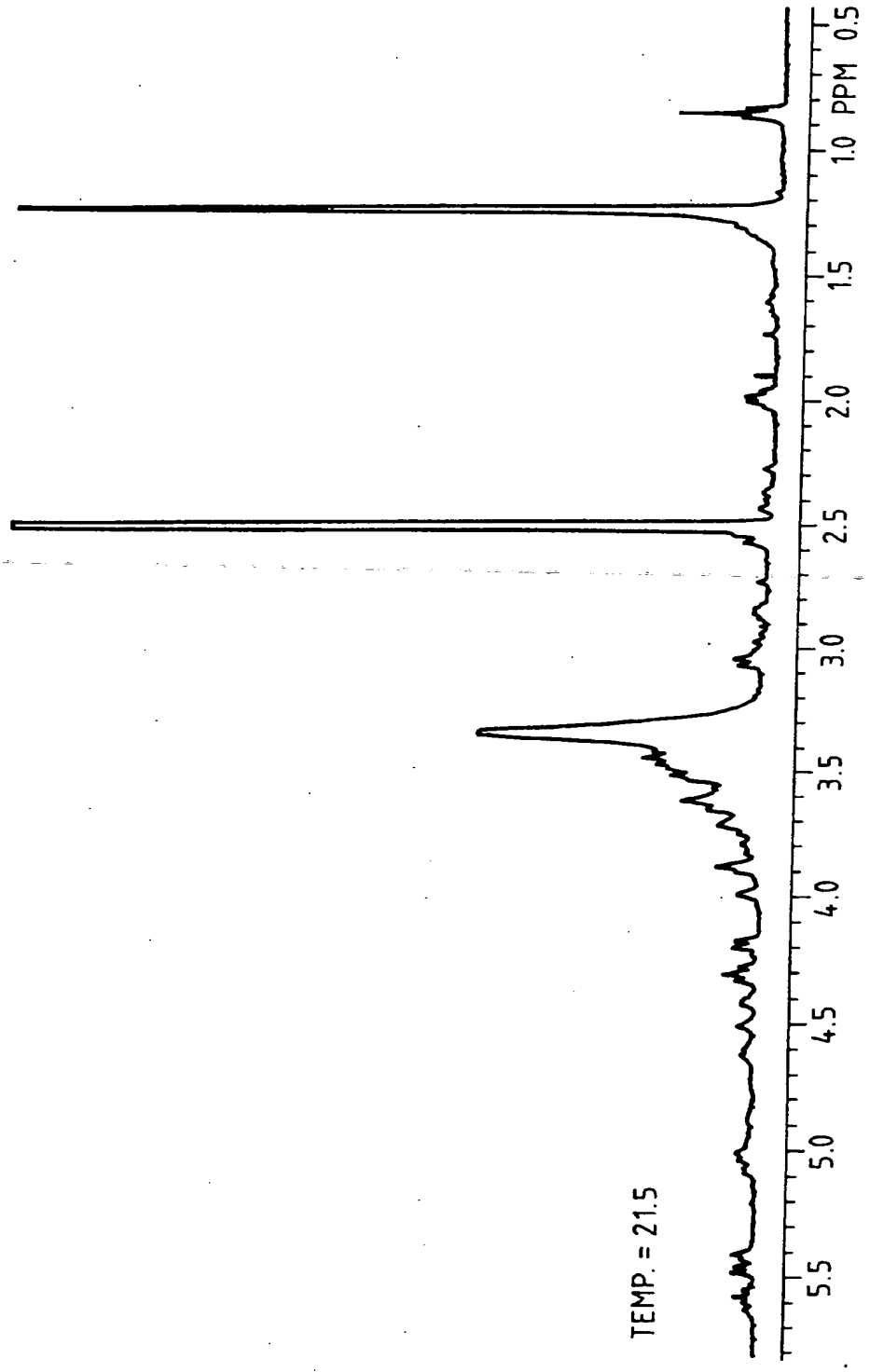
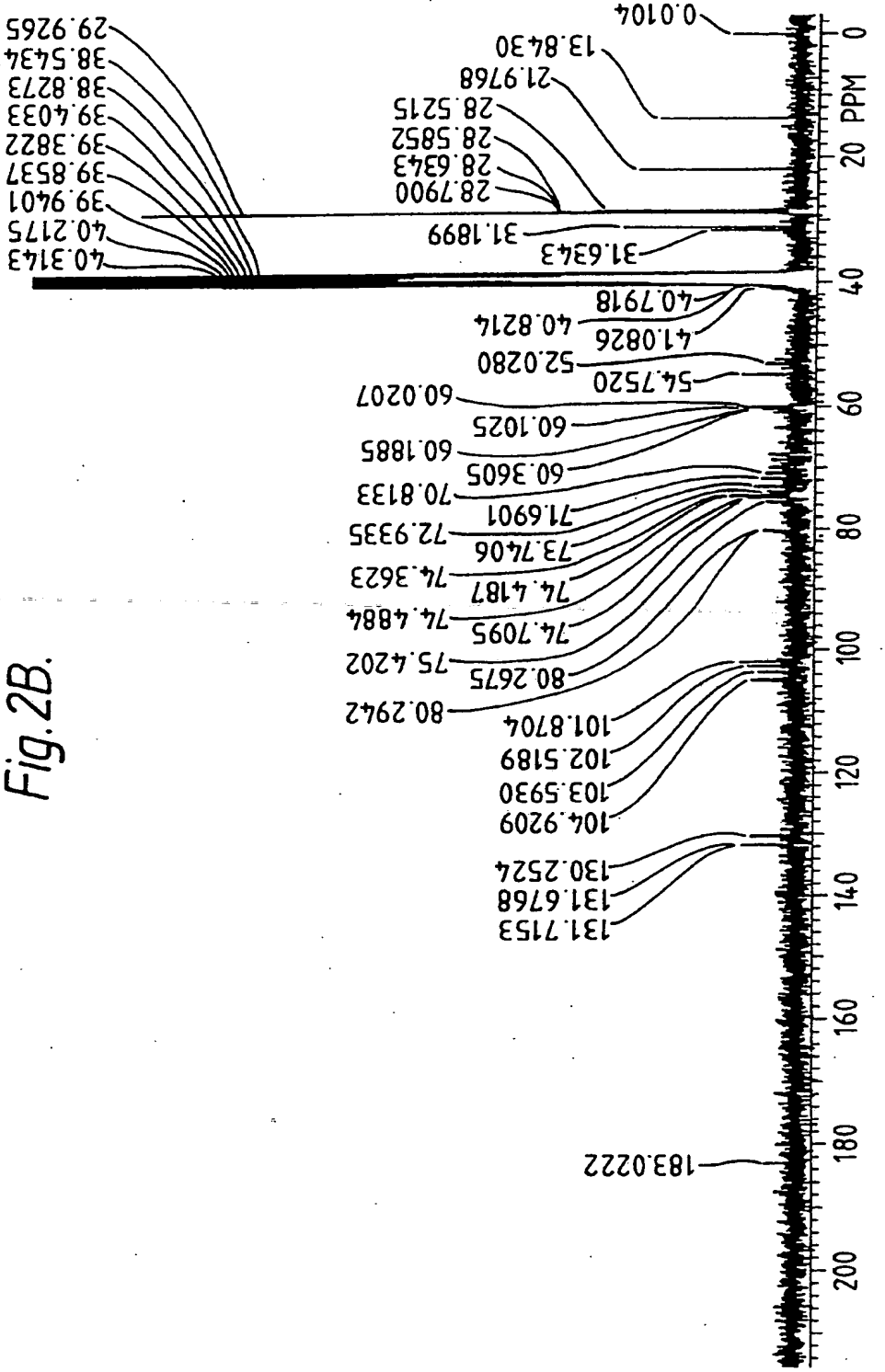


Fig.2A.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41.0826
40.8214
52.0280
54.7520
60.0207
60.1025
60.1885
60.3605
70.8133
71.6901
72.9335
73.7406
74.4187
74.3623
74.7095
74.4884
75.4202
80.2675
80.2942
101.8704
102.5189
103.5930
104.9209
130.2524
131.6768
131.7153
183.0222
29.9265
38.5434
38.8273
39.4033
39.3822
39.8537
39.9401
40.2175
40.3143
0
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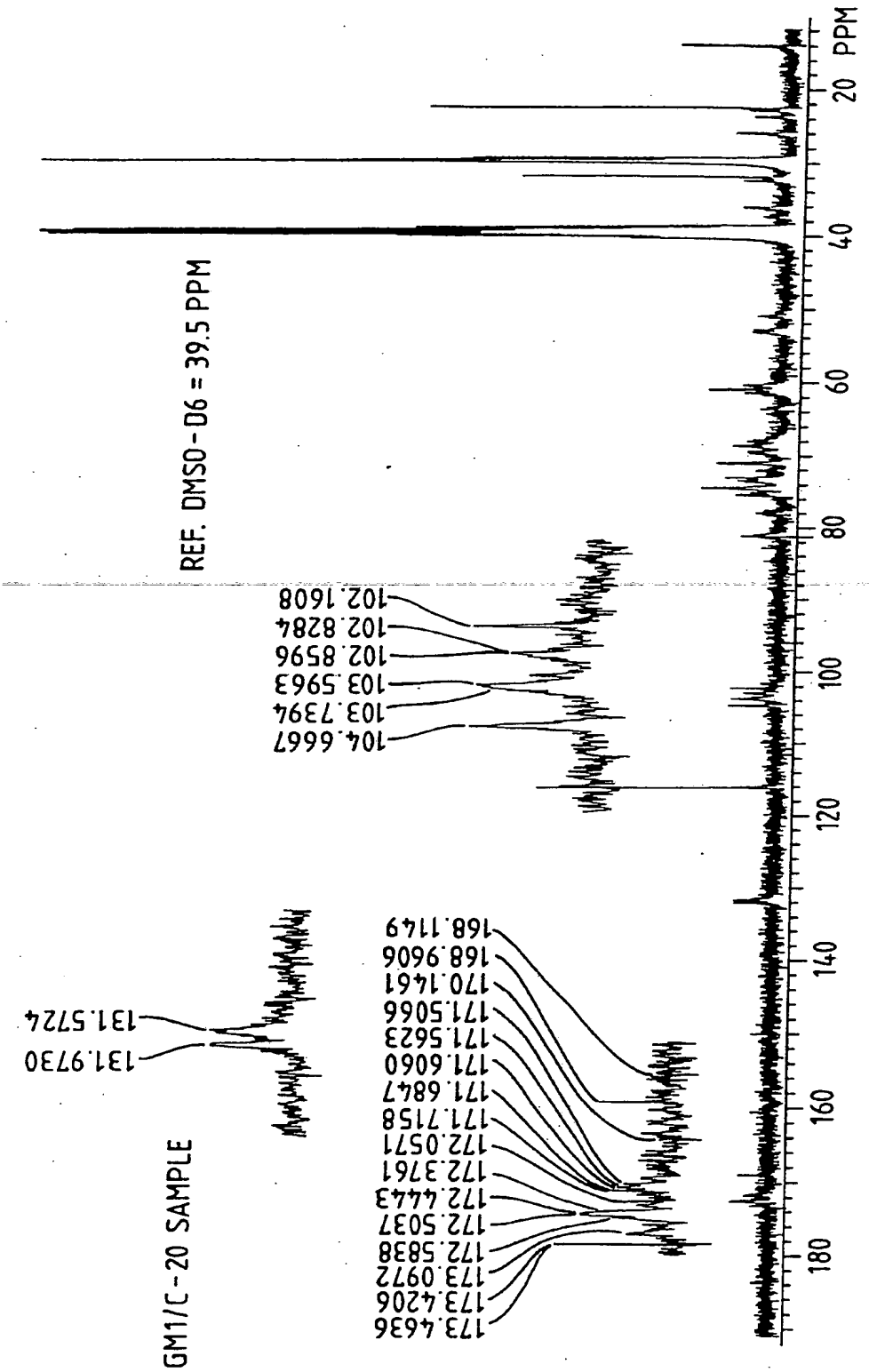
40
60
80
100
120
140
160
180
200

Fig. 2B.

Fig. 3.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40
60
80
100
120
140
160
180
REF. DMSO-D6 = 39.5 PPM
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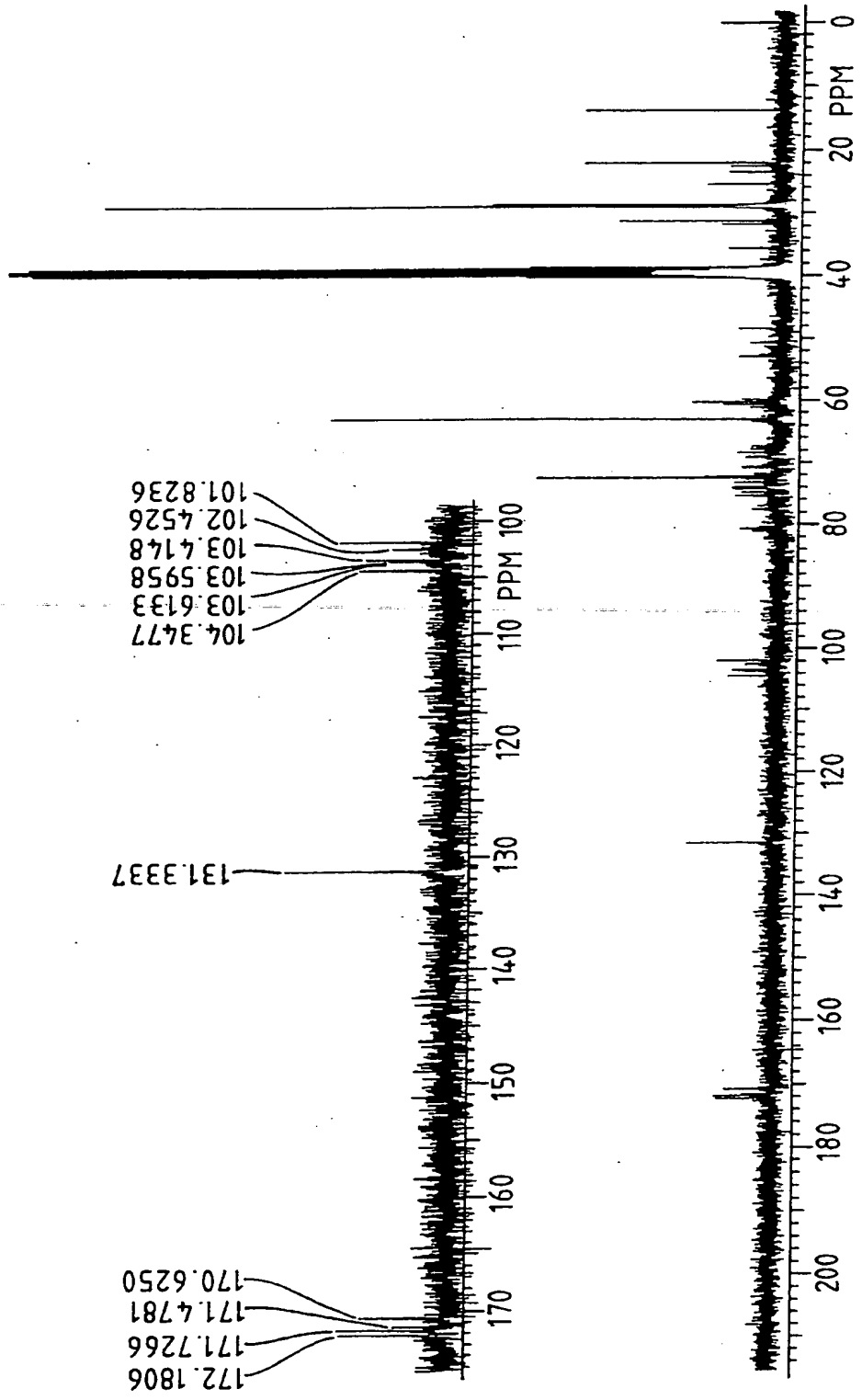
102.8596
103.5963
103.7394
104.6667
131.9730
131.5724
GM1/C-20 SAMPLE
168.1149
168.9606
170.1461
171.5066
171.5623
171.6060
171.6847
171.7158
172.0571
172.3761
172.4443
172.5037
172.5838
173.0972
173.4206
173.4636


0
20 PPM
40
60
80
100
120
140
160
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200
100
110 PPM
120
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160
170
101.8236
102.4526
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170.6250
171.4781
171.7266
172.1806

Fig. 4.

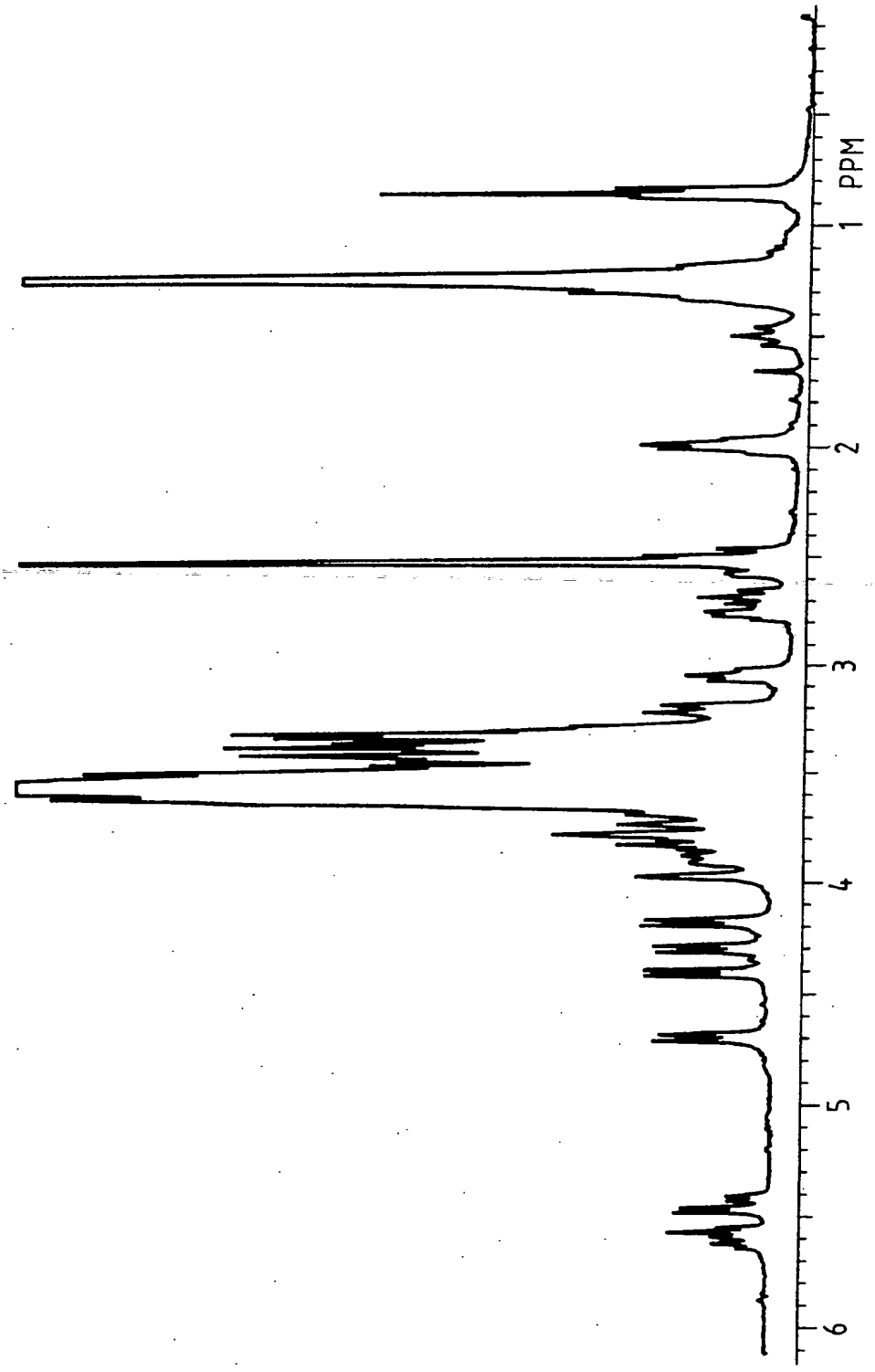


Fig.5.

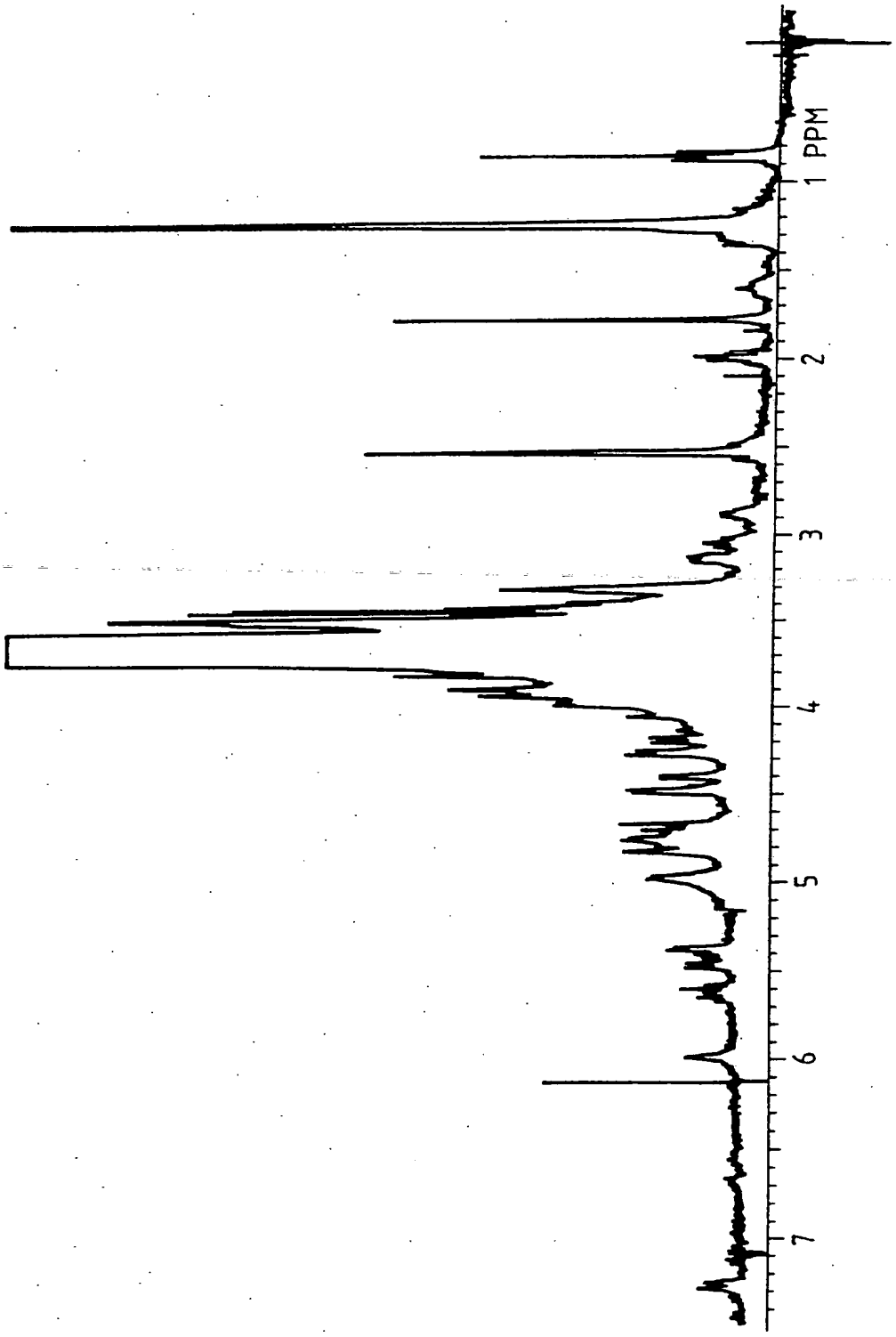


Fig.6.

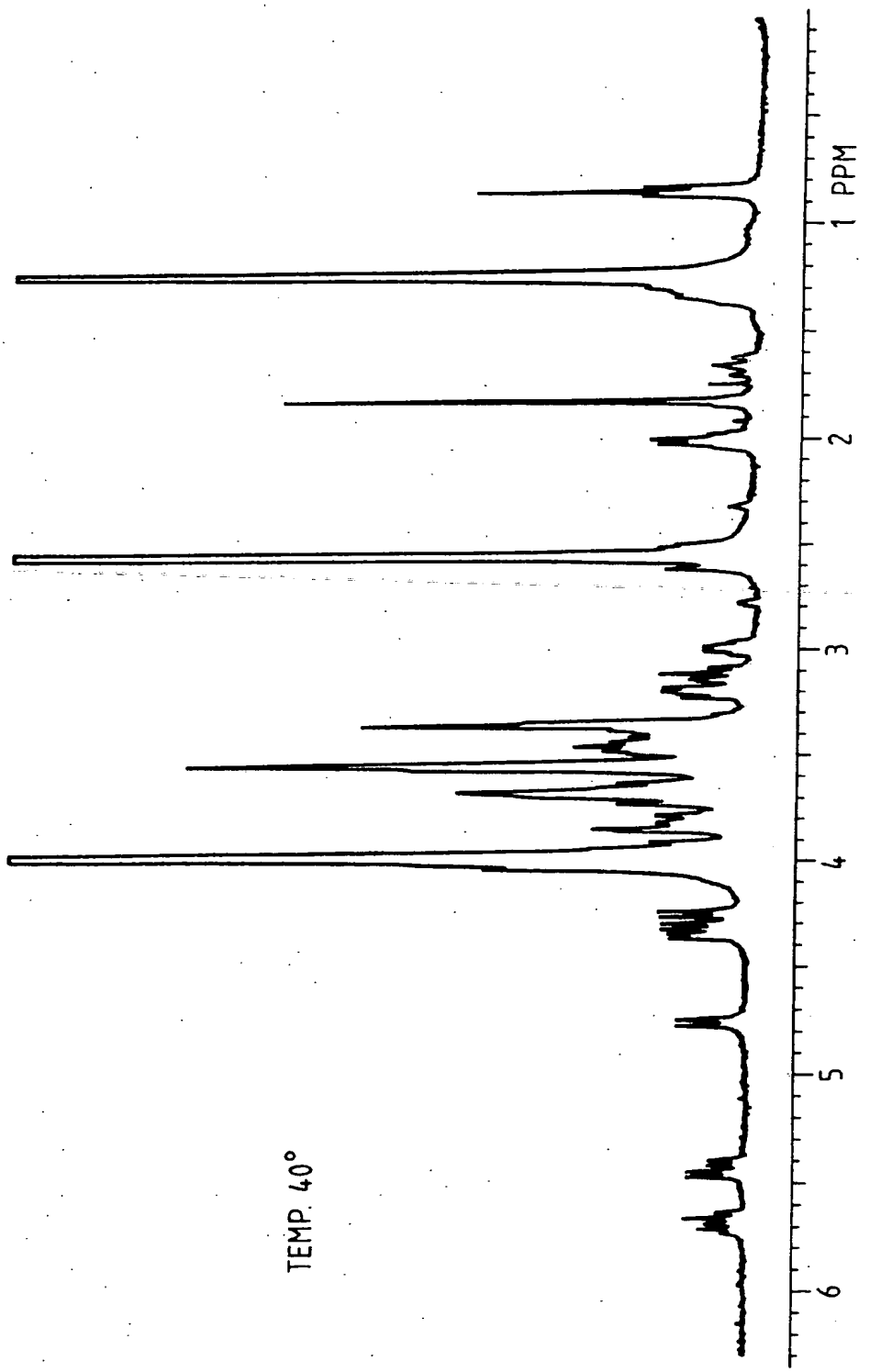


Fig.7.

Fig. 8.

EFFECT OF MONO-N-DEACETYL-LYSO-GM_1 (GM_1L) AND INTERFERONS ON LEUKEMIA L1210 PROLIFERATION

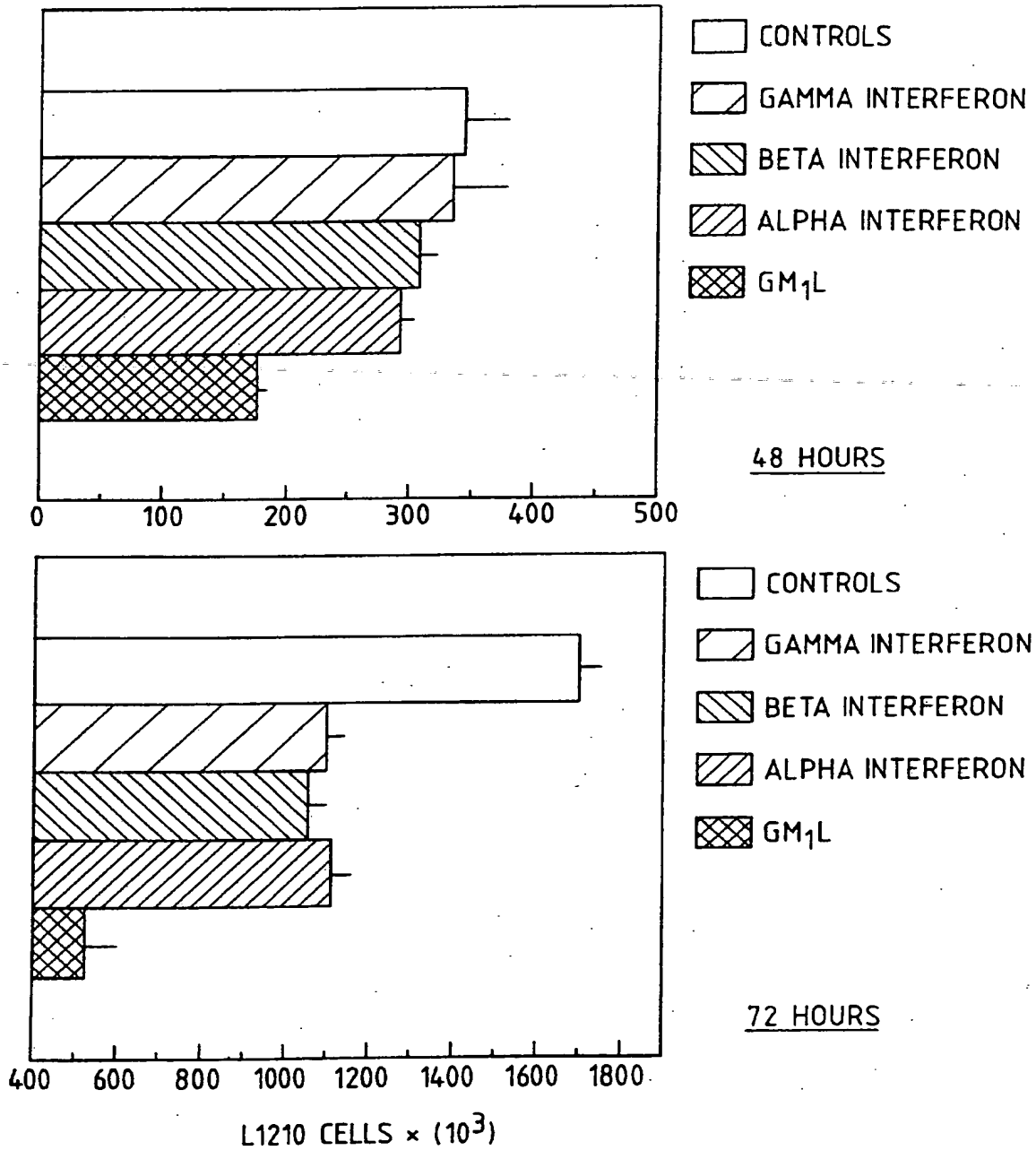


Fig. 9.

EFFECT OF MONO-N-DEACETYL-LYSO-GM_1 (GM_1L) AND INTERFERONS ON DAUDI CELLS PROLIFERATION

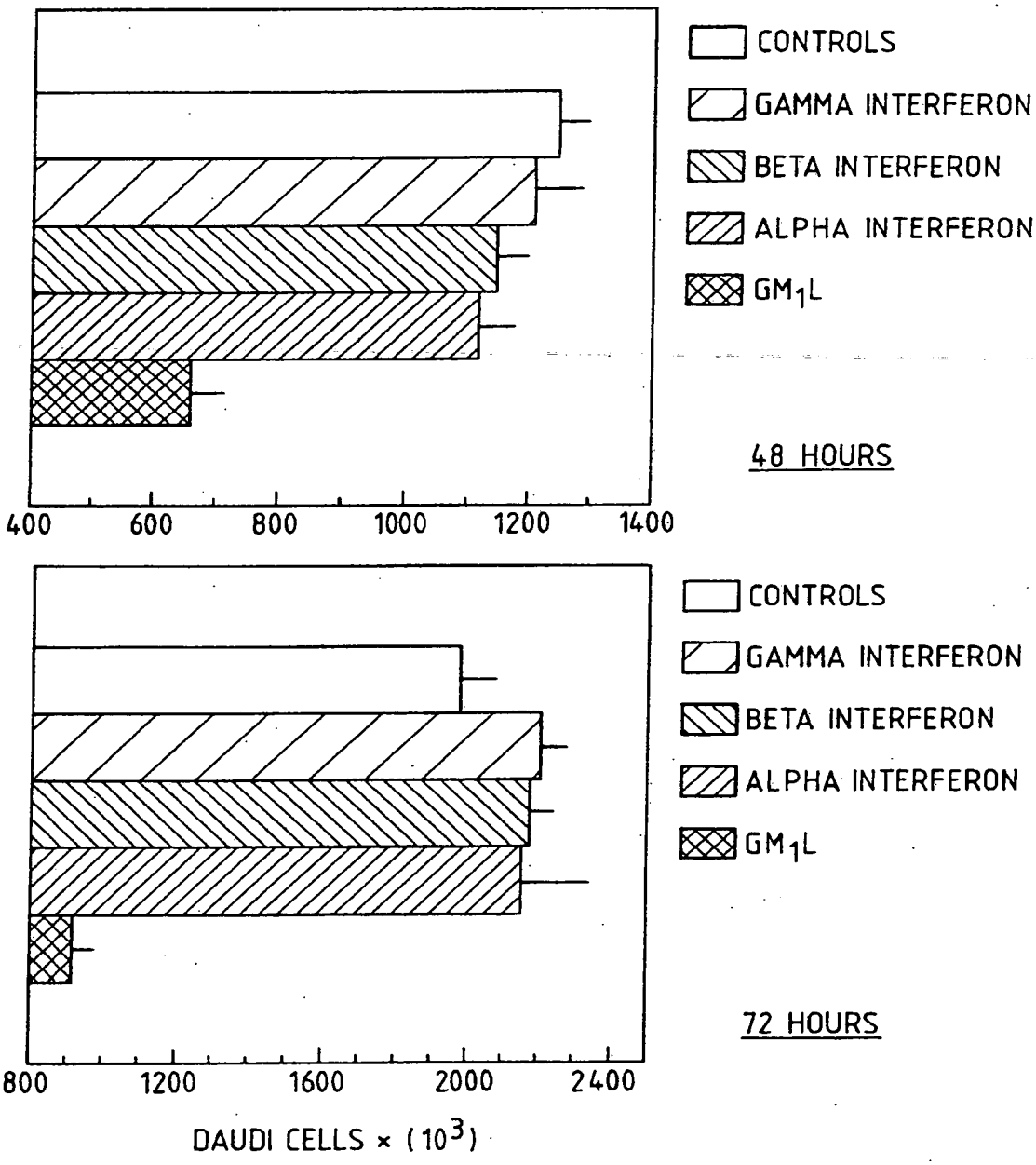


Fig. 10.

EFFECT OF PREINCUBATION OF LEWIS LUNG CARCINOMA CELLS WITH MONO-N-DEACETYL-LYSO-GM_1 (GM_1L) ON THEIR ONCOGENICITY

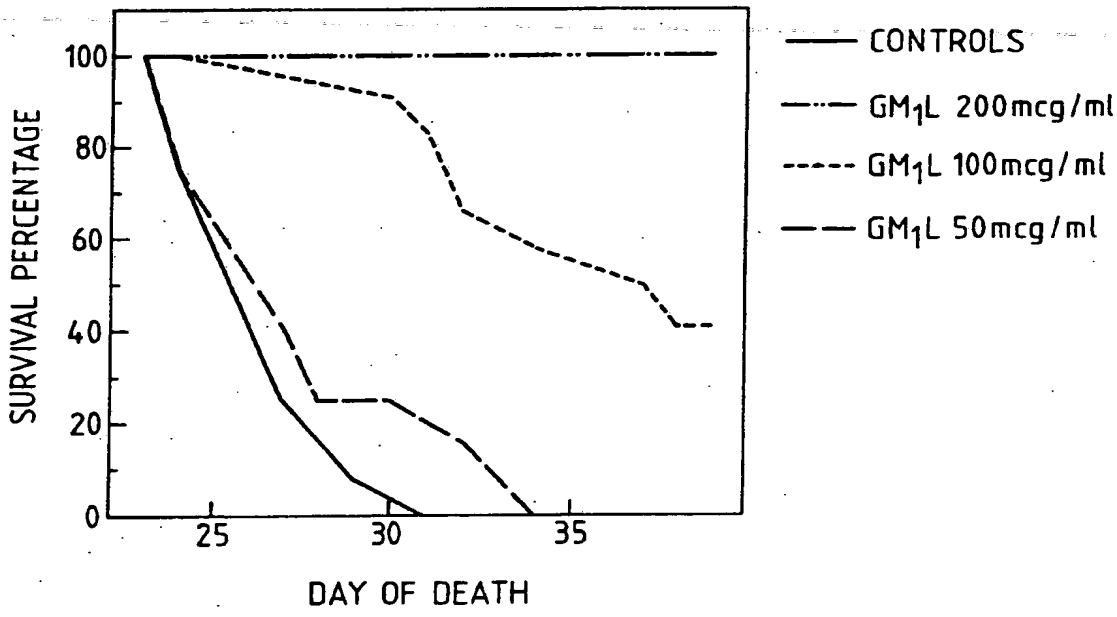
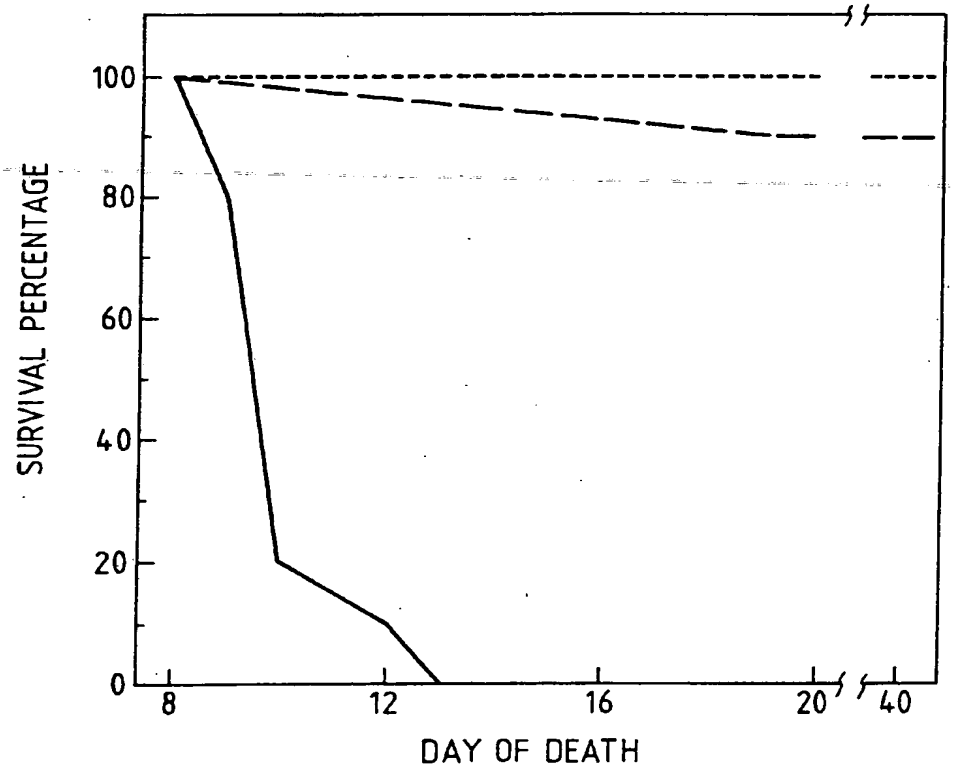


Fig. 11.

EFFECT OF MONO-N-DEACETYL-LYSO-GM_1 (GM_1L) ON LYMPHOMA L 5178Y IN VITRO



——CONTROLS

----GM1L 100mcg/ml

——GM1L 50mcg/ml

Fig. 12. BONE MARROW GRAFT REJECTION EFFECT OF MONO-N-DEACETYL-LYSO-GM_1 (GM_1L)

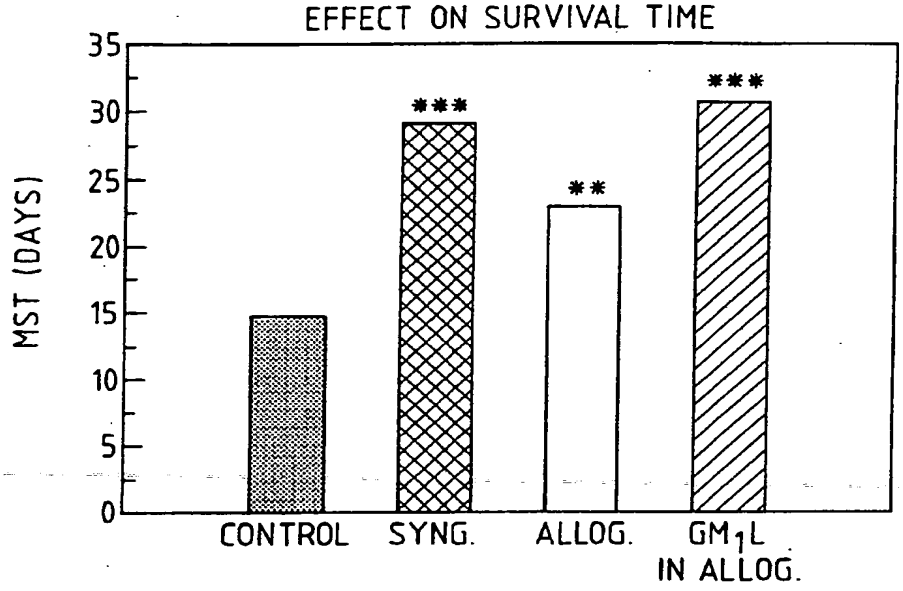


Fig. 13. BONE MARROW GRAFT REJECTION EFFECT OF MONO-N-DEACETYL-LYSO-GM_1 (GM_1L)

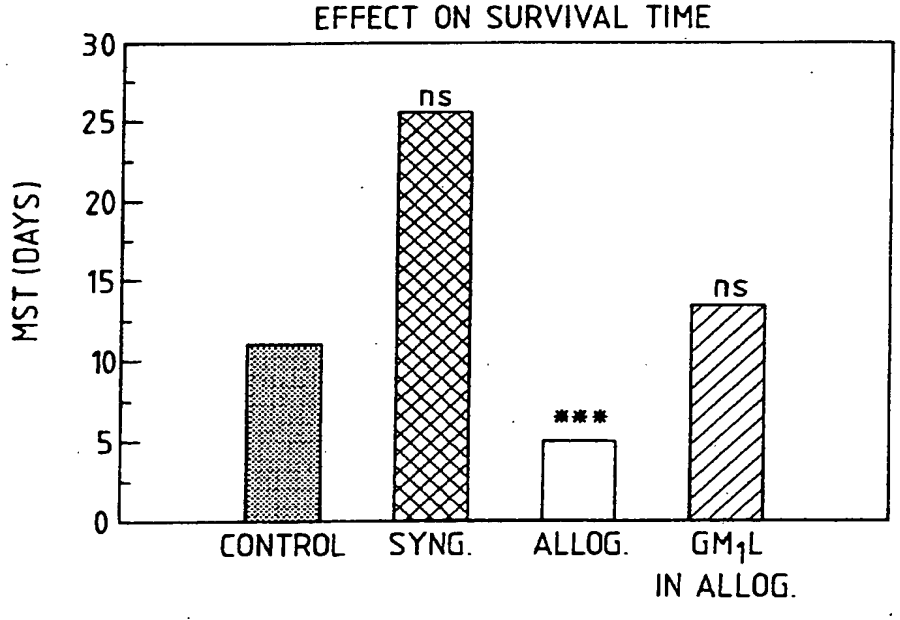


Fig. 14.

EFFECT OF MONO-N-DEACETYL-LYSO-GM_1 (GM_1L) TREATMENT ON MORTALITY DUE TO EAE INDUCTION WITH BOVINE MYELIN IN GUINEA PIGS

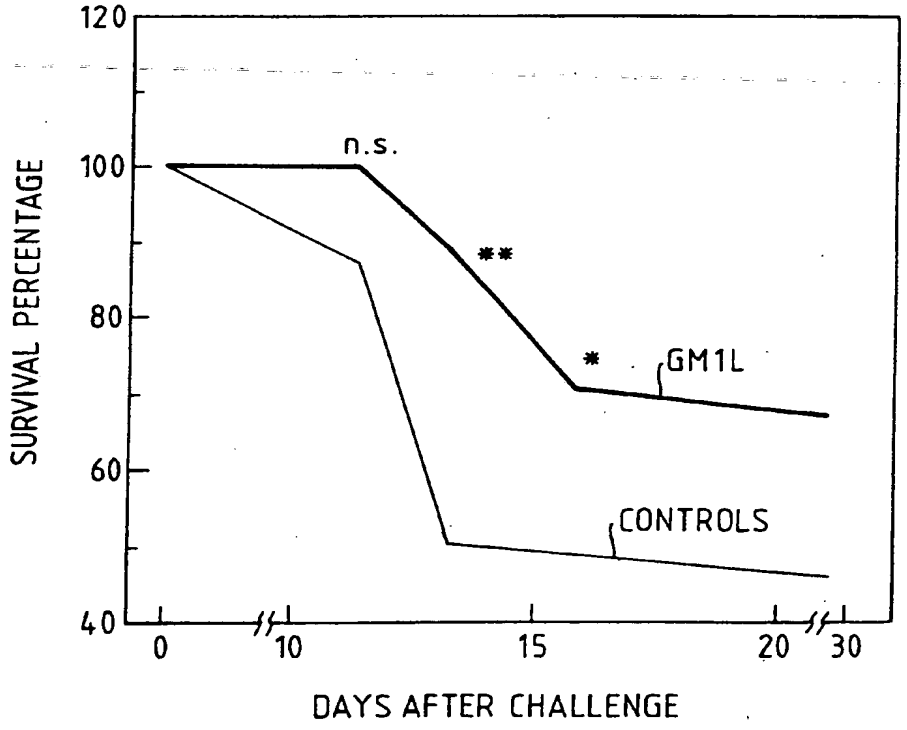
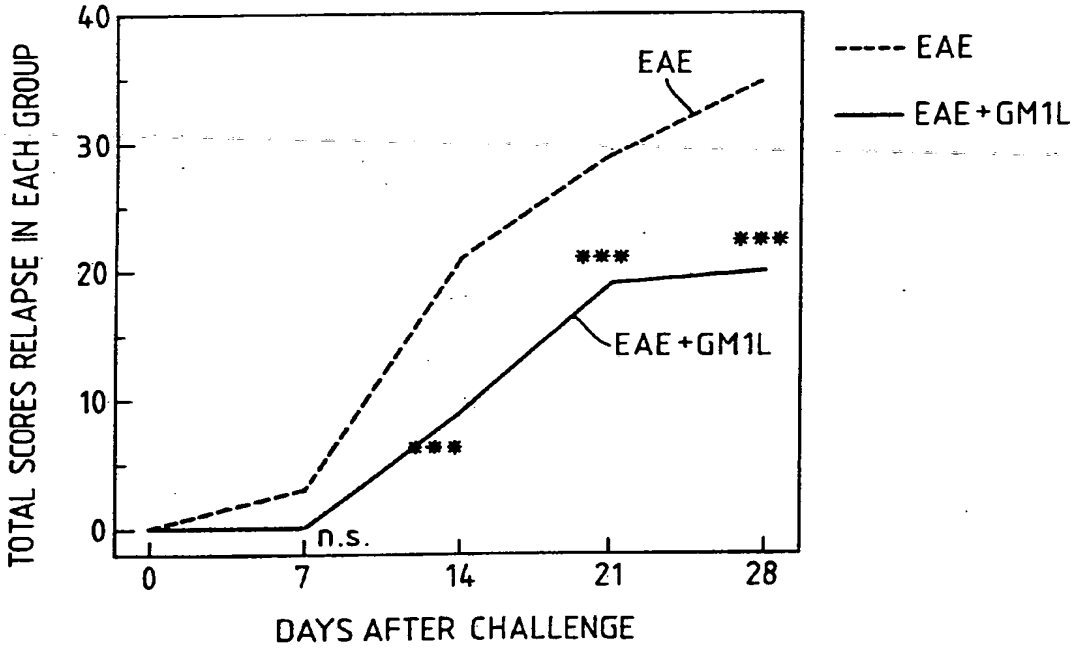


Fig. 15.

PROTECTIVE EFFECT OF MONO-N-DEACETYL-LYSO-GM_1 (GM_1L) ON GUINEA PIGS AFTER EAE INDUCTION (BY BOVINE MYELIN)



CLINICAL SCORES AND CRITERIA FOR RELAPSE:
0-NO SIGNS; 1-WEIGHT LOSS; 2-MILD PARESIS; 3-MODERATE PARESIS; 4-SEVERE PARESIS, AND FAECAL IMPACTION; 5-DEATH

Fig. 16.

EFFECT OF DI-N-DEACETYL-LYSO-GM_1-C_{20} ON HOG PANCREAS PLA_2 ON LIPOSOMES

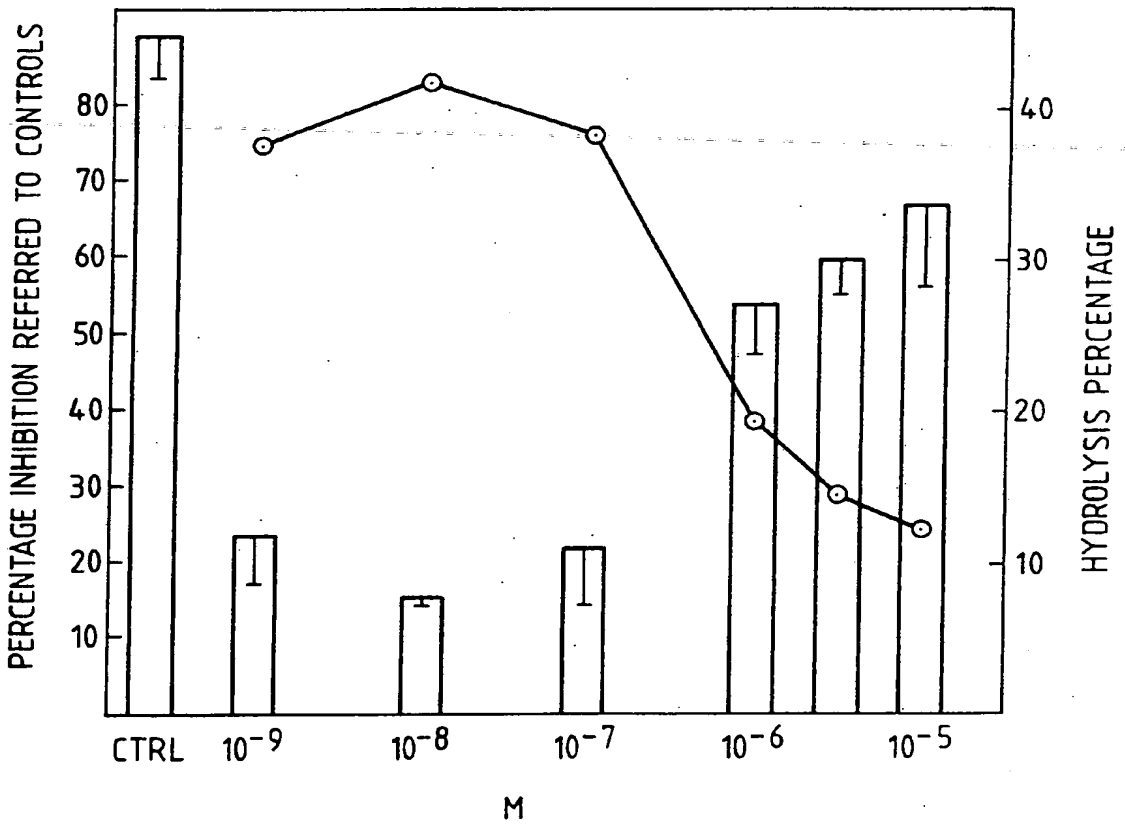
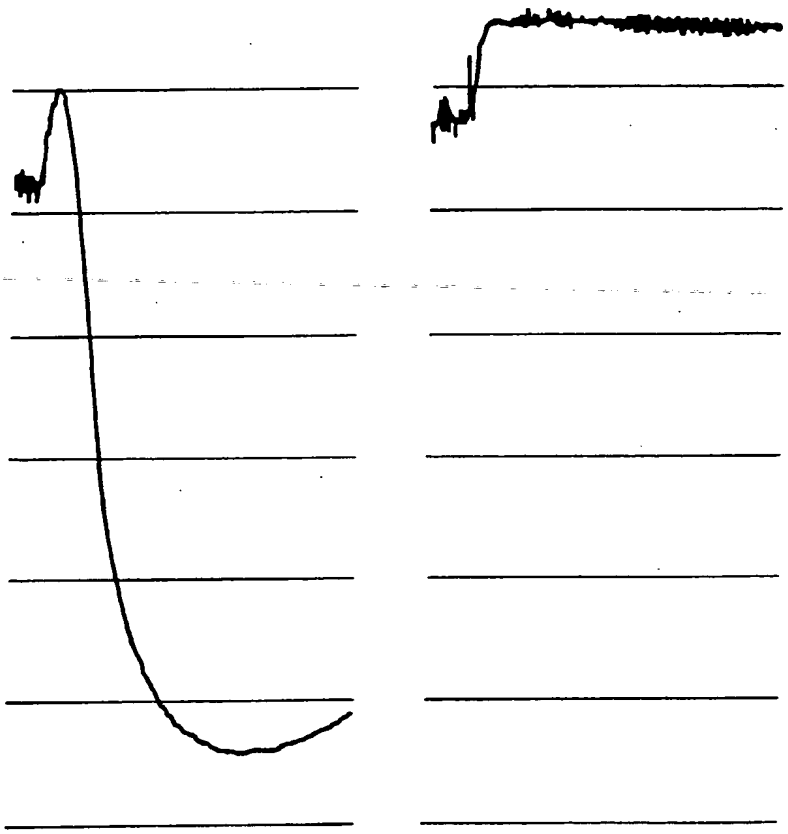


Fig. 17.

EFFECT OF DI-N-DEACETYL-LYSO-GM_1-C_{20} ON RABBIT PLATELET AGGREGATION IN VIVO



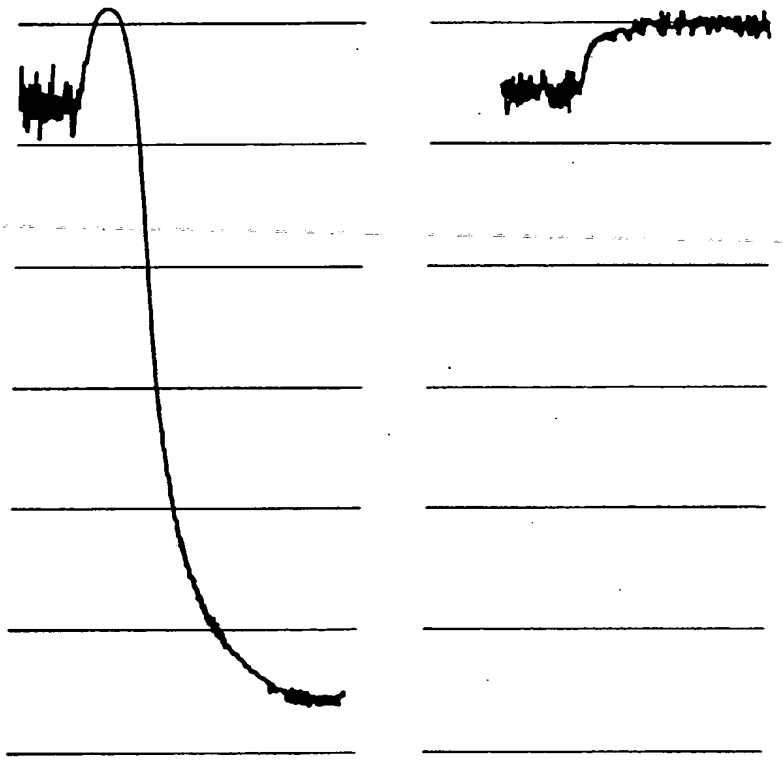
A.A. 3mcl = 45mcg/ml
TIME 0

A.A. 3mcl = 45mcg/ml
>18hrs

DI-N-DEACETYL-LYSO-GM_1-C_{20} AT 0.2 mg/kg p.o.
A.A. = ARACHIDONIC ACID

Fig. 17 (cont.)

EFFECT OF DI-N-DEACETYL-LYSO-GM_1-C_{20} ON RABBIT PLATELET AGGREGATION IN VIVO



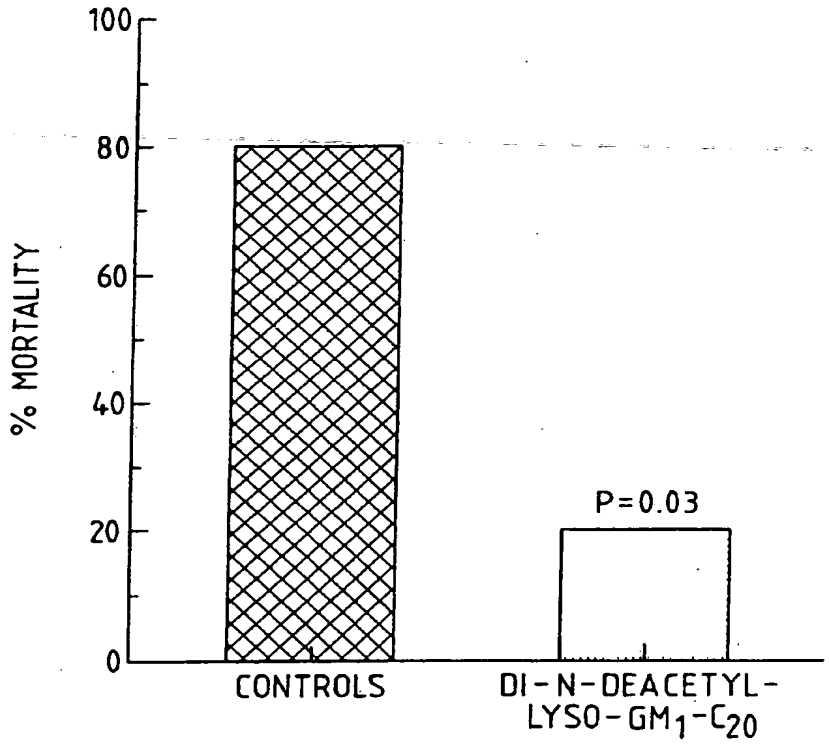
A.A. 3mcl = 45mcg/ml
TIME 0

A.A. 3mcl = 45mcg/ml
>18hrs

DI-N-DEACETYL-LYSO-GM_1-C_{20} AT 0.13mg/kg p.o.
A.A. = ARACHIDONIC ACID

Fig. 18.

EFFECT OF DI-N-DEACETYL-LYSO-GM_1-C_{20} ON THROMBIN-INDUCED THROMBOEMBOLISM IN MICE



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/02028

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^5: A 61 K 31/70, C 07 H 15/10

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System	Classification Symbols
IPC^5	A 61 K 31/00, C 07 H 15/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
X	EP, A, 0328420 (BIOMEMBRANE INSTITUTE) 16 August 1989 see page 6, line 63 - page 7, line 20; claims 31-33,36 --	1,13
X	EP, A, 0321287 (CITY OF HOPE) 21 June 1989 see claims 1,2 --	1
P,X	Chemical Abstracts, vol. 113, no. 1, 2 July 1990, (Columbus, Ohio, US), E.V. Dyatlovitdkaya et al.: "Derivatives of ganglioside GM3 and their immuno-modulating effects", see page 14, abstract 151b & Bioorg. Khim. 1990, 16(3), 402-6 --	1,14,15

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th February 1991	28. 03. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. PEIS M. Peis

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Biochemistry, vol. 24, 15 January 1985, American Chemical Society, S. Neuenhofer et al.: "Synthesis of lysogangliosides", pages 525-532 see page 528, column 2, line 19 - page 531, column 2, line 7 (cited in the application)	7,8

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9002028 SA 43258

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 25/03/91 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0328420	16-08-89	EP-A- 0332298	13-09-89
		JP-A- 2104594	17-04-90
EP-A- 0321287	21-06-89	AU-A- 2700788	22-06-89
		JP-A- 1279837	10-11-89